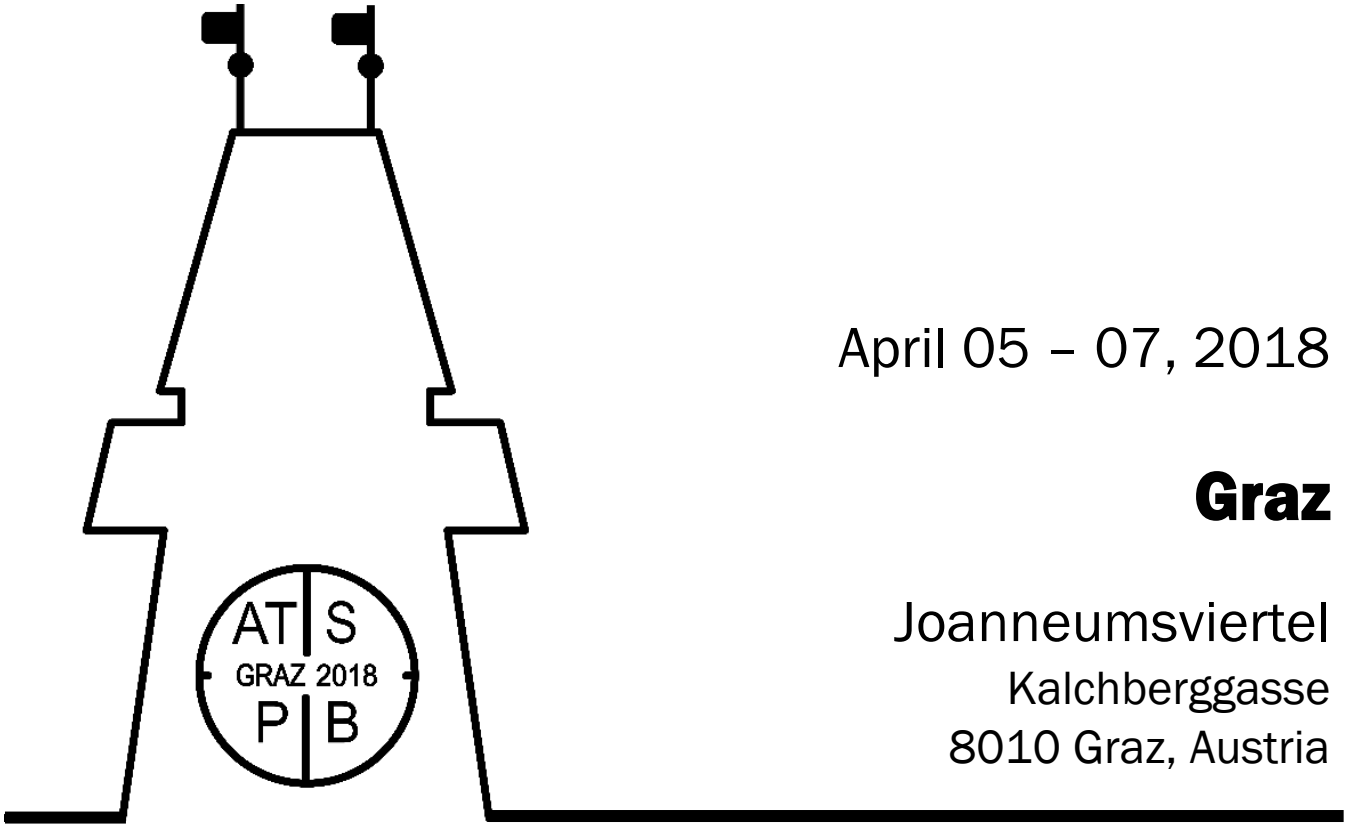
Conference Book

22nd Meeting

Austrian Society of Plant Biology (ATSPB)



April 05 – 07, 2018

Graz

Joanneumsviertel
Kalchberggasse
8010 Graz, Austria

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Austrian Society of Plant Biology (ATSPB)

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ATSPB Board
Ilse Kranner
Gilbert Neuner, Johanna Wagner
Department of Botany, University of Innsbruck

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Alexandra Jammer, Stephan Monschein, Hartwig W. Pfeifhofer, Edith Stabentheiner, Tomáš Werner, Günther Zellnig
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22nd ATSPB Meeting

Conference Book

Layout & Graphics
Alexandra Jammer

Content

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Program

Thursday, April 05, 2018

11:00 **Start of registration**

13:50 – 14:00 **Opening ceremony**
Ilse Kranner, ATSPB president
Maria Müller, head of organizing committee

14:00 – 15:40 **Talks, Session I: Abiotic Stress**
(Chair: Ingeborg Lang)

14:00 – 14:20 Markus Teige
Chloroplast Ca^{2+}- signaling in development and stress response

14:20 – 14:40 Jakob Weiszmann ♦
The role of vacuolar invertase in shaping photosynthetic stress response of *Arabidopsis thaliana*

14:40 – 15:00 Maria Köhler ♦
Analysis of the role of galactinol in abiotic stress responses

15:00 – 15:20 Ján Kováč ♦
Deposition of lignin and suberin into root tip as a reaction to copper exposure and stress

15:20 – 15:40 Marketa Absolonova ♦
Surface pH changes and salinity response of *Chara australis*

15:40 – 16:10 **Coffee Break**

16:10 – 17:30 **Talks, Session II: Plant Molecular Biology**
(Chair: Raimund Tenhaken)

16:10 – 16:30 Tomáš Werner
The role of the endoplasmic reticulum in regulating cytokinin responses

16:30 – 16:50 Andreas Bachmair
Protein modification and plant responses to the environment

16:50 – 17:10 Julia Richter ♦
The cell wall as platform for heavy metal sensing

17:10 – 17:30 Doris Lucyshyn
The role of O-glycosylation in plant developmental transitions

17:30 – 19:00 **Poster Session**
and **Beer Degustation** (presented by Julia Jamnig and René Rehorska)

Friday, April 06, 2018

09:00 – 10:20 **Talks, Session III: Plant Physiology I**
(Chair: Tomáš Werner)

09:00 – 09:20 Raimund Tenhaken
Arabinokinase, a unique fusion protein involved in arabinose toxicity, is challenging the cellular energy homeostasis kinases

09:20 – 09:40 Klaus Herburger ♦
Significance of hetero-trans-β-glucanase in cell wall metabolism of *Equisetum* and transgenic *Arabidopsis*

09:40 – 10:00 Anna Gasperl ♦
PGI and 1-FEH activities, a key combination of physiological markers from primary carbohydrate and fructan metabolism to breed high sugar grasses

10:00 – 10:20 Lisa Fürtauer ♦
Quantifying the subcellular plant metabolome

10:20 – 10:50 **Coffee Break**

10:50 – 12:10 **Talks, Session IV: Plant Physiology II**
(Chair: Ilse Kranner)

10:50 – 11:10 Gábor Kocsy
Control of photosynthesis, glutathione and amino acid metabolism by light quantity and quality in wheat

11:10 – 11:30 Verena Ibl
Bridging proteomics, RT-qPCR and microscopy to unravel the spatio-temporal expression changes of hordoindolines across development-dependent changes in barley grains

11:30 – 11:50 Michal Goga ♦
Localization and identification of calcium oxalate forms in lichens

11:50 – 12:10 Wolfram Weckwerth
Stress signaling networks in algae and plants

12:10 – 14:00 **Poster Session and Lunch**

14:00 – 15:40 **Talks, Session V: Cell Biology**
(Chair: Ursula Lütz-Meindl)

14:00 – 14:20 Philip Steiner ♦
Ionic stress induces fusion of mitochondria to 3-D networks: an electron tomography study

14:20 – 14:40 Marieluise Weidinger
Structure and physiology of giant chloroplasts in the deep shade adapted lycopod *Selaginella erythropus*

14:40 – 15:00 Dominik Harant ♦
The cortical endoplasmic reticulum before and after plasmolysis in *Physcomitrella patens*

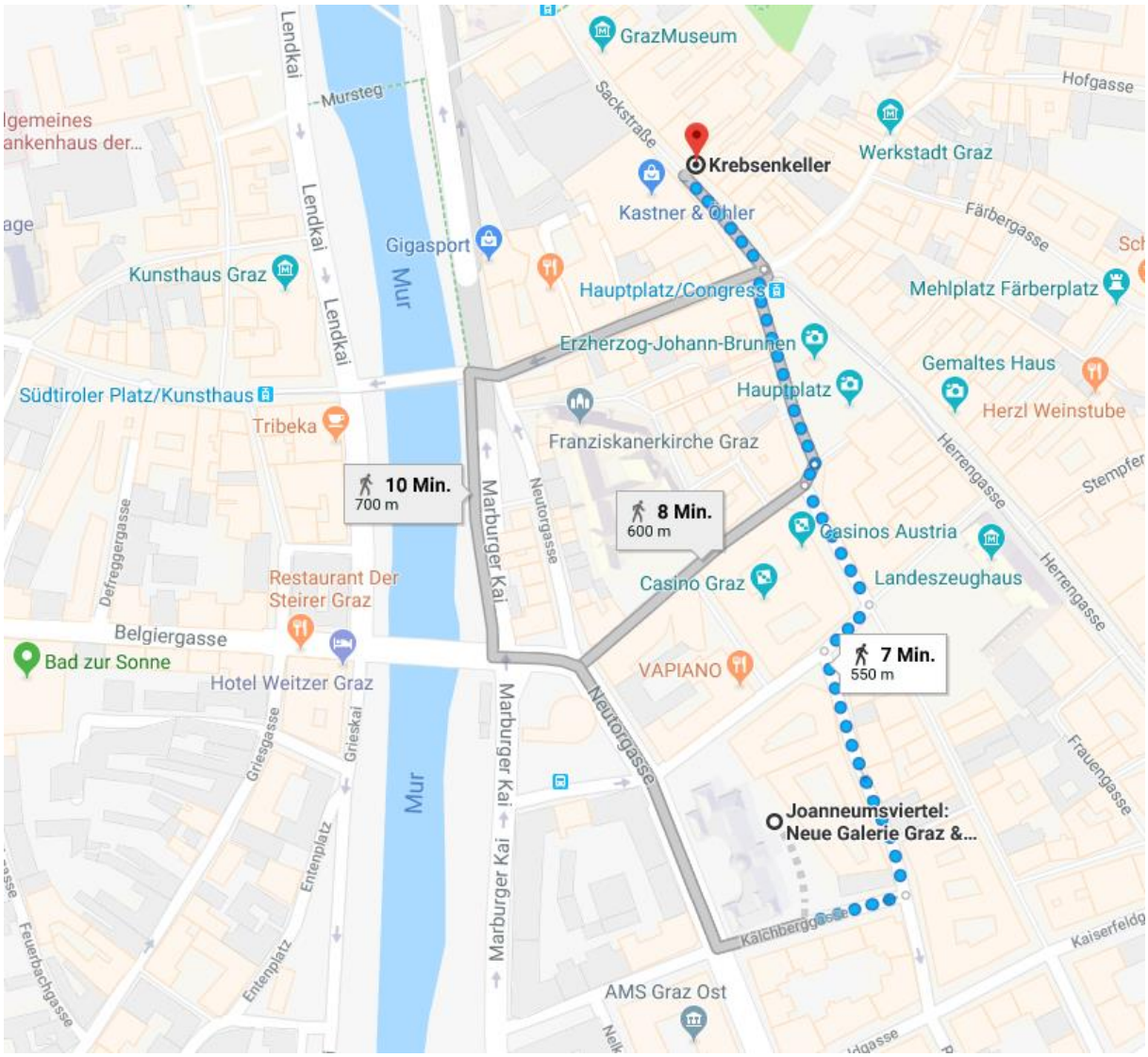
15:00 – 15:30 **Coffee Break**

15:30 – 17:00 **Guided Tours through the Museum**

17:15– 18:30 **ATSPB General Assembly**

19:00 **Conference Dinner at Krebsenkeller**
(approx. 10 min walk)

Gasthof Krebsenkeller
Sackstraße 12
8010 Graz
www.krebsenkeller.at



Saturday, April 07, 2018

09:15 – 10:15 **Talks, Session VI: Plant Microbe Interaction**
(Chair: Andreja Urbanek Krajnc)

09:15 – 09:35 Armin Djamei
Dissecting the effectome of the maize pathogen *Ustilago maydis*

09:35 – 09:55 Davide Gerna ♦
Selective modification of the wheat seed microbiota affects hydrogen peroxide production in wheat seedlings

09:55 – 10:15 Alexandra Jammer
Marker-assisted selection for Tomato Mosaic Virus resistance in local varieties of *Solanum lycopersicum* L.

10:20 – 10:50 **Coffee Break**

10:50 – 11:30 **Talks, Session VII: Ecophysiology**
(Chair: Alexandra Jammer)

10:50 – 11:10 Erwann Arc
Interactive effects of biotic and abiotic stress factors during seed production on *Helianthus annuus* seed quality

11:10 – 11:30 Andrea Ganthaler ♦
Complex patterns in xylem hydraulics of subalpine dwarf shrubs

11:30 – 12:00 **Meeting of the Awards Committee**

12:00 – 12:30 **Awards Ceremony** (Best Talk, Best Poster), **Closing Remarks**

13:30 – ~18:00 **Excursion**
for details, see description on page 15

♦♦♦ Awards: Best Poster and Best Talk presented by Students or Junior PostDocs ♦♦♦

At the end of the conference, the ATSPB will award a prize each for the best poster and best talk presented by early career scientists (students, junior PostDocs; marked by symbol ♦). As a participant of this meeting, either you will be eligible for a prize, or you will be asked to vote for your favorite talks and posters.

If you are asked to vote: Please write the names of the presenting authors of the two best posters and the two best talks on the voting sheets you will be handed at registration. Drop the voting sheet for the posters in the voting box after the lunch poster session on Friday. Drop the voting sheet for the talks in the voting box after the last session on Saturday.

An Awards Committee will count the votes and make the final decision about the award-winning presentations in a meeting after the final session. The members of the committee are Marie-Theres Hauser, Alexandra Jammer, Ilse Kranner, Ingeborg Lang, Maria Müller, Raimund Tenhaken, Andreja Urbanek Krajnc, and Tomáš Werner.

List of Poster Presentations
(in alphabetical order)

Poster 1 **Sebastian J. Antreich ♦**
Lignification of endocarp cells during the development of the walnut fruit

Poster 2 **Margret Eckhard ♦**
A fast cryo-preservation technique of *Physcomitrella patens* for element analysis in the electron microscope

Poster 3 **Reinmar S. Eggers, Bastian Daniel ♦**
In vivo and in vitro characterization of monolignol oxidoreductases from the berberine bridge enzyme-like protein family in *Arabidopsis thaliana*

Poster 4 **Ilse Foissner**
Constitutive endocytosis in *Chara* internodal cells: comparison between plasma membrane dyes and fluid phase markers

Poster 5 **Gábor Galiba**
Transcript and phytohormone analysis based prediction of genotype-specific biological processes in cold-treated wheat

Poster 6 **Karina Eva Hauer ♦**
Cellulases and pectic enzymes – the armoury of plant pathogen *Colletotrichum coccodes*?

Poster 7 **Assiyeh Hamidipour ♦**
Investigating enzyme activity in some *Pinguicula* species

Poster 8 **Mihaela Jancar ♦**
Plant pathogen interactions of *Fusarium ssp*. and *Armoracia rusticana* and possibilities for prevention

Poster 9 **Ursula Ladinig**
Vegetative and reproductive performance along a climatic gradient does not explain GP in *Ranunculus kuepferi*

Poster 10 **Stephan Manhalter ♦**
Ni tolerance and its distinguished amelioration by chelating agents in *B. napus* cultivars

Poster 11 **Margaréta Marcinčinová ♦**
Calcium and iron content in biofilms of active travertine springs in Slovakia

Poster 12 **Gilbert Neuner**
Deep supercooling of buds of *Alnus alnobetula*: surface impregnation with lipophilic substances allows innocuous growth of ice masses between leaf primordia

Poster 13 **Gregor Pichler ♦**
Phytohormone release by the optionally lichenized alga *Coccomyxa sp.*

Poster 14 **Wolfgang Punz**
Cold air talus sites in the Eastern Alps

Poster 15 **Kerstin Reibenschuh ♦**
Can sulfur fertilization improve the defense reaction of the Styrian oil pumpkin after *Didymella bryoniae* treatment?

Poster 16 **Thomas Roach**
Distinguishing the mechanistic contributions to NPQ in *Chlamydomonas reinhardtii*

Poster 17 **Valentina Romagna ♦**
Heavy metal uptake via endocytosis during vesicle turnover in growing root hairs of *Triticum aestivum*

Poster 18 **Dajana Ručová ♦**
Does usnic acid influence ploidy levels in mosses?

Poster 19 **Nadia Sasani ♦**
Impact of drought stress on the cell wall design of larch trees

Poster 20 **Sarah-Salome Sidra ♦**
Yeast in bioremediation – experiments with *A. arenosa* on zinc contaminated soils

Poster 21 **Matthias Stegner ♦**
Testing viability of plant cells: can loss of turgor potential signalize cell death?

Poster 22 **Gerhard Strauß ♦**
Trichomes on flowers of *Lavandula angustifolia*

Poster 23 **Lisa Marie Strobl ♦**
Sensitivity loss of *Venturia inaequalis* against the plant protection product Delan WG and its effect on the germination behaviour of the conidia

Poster 24 **Polona Sušnik ♦**
Following mulberry (*Morus sp.*) footprints: Geographic distribution and diversity of leaf morphology of heritage trees in Slovenia

Poster 25 **Larissa Teuschler ♦**
Microscopic-analytical characterization of different sweet potato varieties (*Ipomoea batatas*)

Poster 26 **Katrin Thiering ♦**
The organization of some subcellular structures in the model moss *Physcomitrella patens*

Poster 27 **Marina Toplak ♦**
Uncovering the role of the single berberine bridge enzyme homolog of *Physcomitrella patens*

Poster 28 **Tina Ugulin ♦**
Morphometric analyses of phytoliths in different mulberry genotypes

Poster 29 **Johanna Wagner**
Winter snow cover is essential for flower bud survival in high mountain plants

Poster 30 **Angelika Waibel ♦**
Reduced sensitivity of *Venturia inaequalis* against a multi-site fungicide tested in different apple orchards in Styria, Austria

Poster 31 **Nannan Xiao ♦**
Unravelling hierarchical microstructure and chemical changes of walnut shells

Excursion

guided by **Bernhard Hubmann** (Institute of Earth Sciences, University of Graz)

Saturday, April 07, 2018, 13:30 – ~18:00
(please bring sturdy shoes for walking)

„Südoststeirisches Paläogemüse an Vulkangestein“

Sedimente des geologisch relativ jungen (ca. 19 Mio. Jahre), bis 3000 m tiefen Oststeirischen Beckens dokumentieren eine Füllungsgeschichte, die den Wechsel eines limnisch-fluviatilen zu einem vollmarinen Ablagerungsraum mit anschließender Aussüßungsphase und einer erneuten limnisch-fluviatilen Landschaft erkennen lässt. Diese Entwicklung wird von zwei vulkanischen Phasen (vor ca. 17 – 12 und vor ca. 2,6 – 1,7 Mio. Jahren) begleitet. Die Exkursion wird Blitzlichter auf die sedimentäre und botanische Entwicklung während des Pannoniums (vor ca. 10 – 11 Mio. Jahren) und auf die pliozäne Vulkantätigkeit (vor ca. 2 Mio. Jahren) werfen.

„Paleo vegetables and volcanic rock, served in South-Eastern Styria“

From a geologist’s point of view, the South-East Styrian basin, which is up to 3000 m in depth, is a relatively young area (approx. 19 million years). The landscape has been formed by several phases of sedimentation, changing from a fluvial to a marine depositional environment, and back to a fluvial landscape. In addition, the area was shaped by two phases of volcanic activity (approx. 17 – 12 and approx. 1.6 – 1.7 million years ago). The excursion will focus on selected aspects of the geological and botanical development of the area during the Pannonium (approx. 10 – 11 million years ago) and on the volcanic activity during the Pliocene (approx. 2 million years ago).

Glyptostrobus europaeus (BRONGNIART, 1833) UNGER, 1850. © S. Monschein.

Abstracts

Talks

(in the order of the program)

Chloroplast Ca^{2+}- signaling in development and stress response

M. Leonardelli(1), S. Stael(2), P. Kmiecik(1), V. Roustan(1), M. Grieco(1), U. Vothknecht(3), M. Melzer(4), W. Weckwerth(1), **M. Teige**(1)

(1) University of Vienna, Ecogenomics and System Biology (MOSYS), Althanstrasse 14, 1090 Wien
(2) VIB Gent, Dept. of Plant Systems Biology, Gent University Technologiepark 927, 9052 Gent, Belgium
(3) LMU Munich, Dept of Biology 1, Großhaderner Str. 2-4, 82152 München, Germany
(4) IPK Gatersleben, Structural Cell Biology, Corrensstrasse 3, 06466 Gatersleben, Germany

Calcium is an important secondary messenger in plant signaling and chloroplasts are able to store large amounts of calcium. However, not much is known about the role of calcium in these organelles except for its role as stabilizer of the oxygen-evolving complex at photosystem II (PSII). The free Ca^{2+} concentration in chloroplasts can vary considerably, calling for an involvement of calcium binding proteins [1]. As high concentrations of free Ca^{2+} inhibit CO_2 fixation, it is plausible that calcium binding proteins play a key role in regulating calcium homeostasis in the chloroplast. We have identified two non-characterized chloroplast proteins [2], and show here that these are not only localized in the chloroplast stroma and bind Ca^{2+}, we demonstrate further that they have a strong effect on chloroplast development, photosynthesis, and thylakoid protein phosphorylation. Double knockout or overexpressor plants exhibit slow growth and chlorosis under normal growth conditions as well as altered chloroplast ultrastructure. Moreover, in vivo analysis revealed that PSII is constitutively damaged in double-knock-out lines, while both overexpressors as well as double knock-out lines showed a reduced photosynthetic electron transfer rate and altered thylakoid protein phosphorylation patterns. Unbiased shot-gun proteomics of *lena,b* mutants revealed strongest changes in the levels of photosynthetic proteins and indicate furthermore that retrograde signaling involving GUN proteins is altered in these mutants. In summary, these data suggest that LENA and LENB proteins play an important role in the regulation of chloroplast development, photosynthesis and response to biotic and abiotic stress [3, 4].

[1] Stael S, Wurzinger B, Mair A, Mehlmer N, Vothknecht UC, Teige M. (2012) J. Exp. Botany 63, 1525-1542.
[2] Bayer RG, Stael S, Csaszar E, Teige M. (2011) Proteomics 11(7):1287-99.
[3] Stael S, Kmiecik P, Willems P, Van Der Kelen K, Coll NS, Teige M, Van Breusegem F. (2015) Trends Plant Sci. 20(1):3-11.
[4] Kmiecik P, Leonardelli M, Teige M. (2016) J Exp Bot. 67(13):3793-807.

The role of vacuolar invertase in shaping photosynthetic stress response of *Arabidopsis thaliana*

J. Weiszmann(1,2), L. Fürtauer(1), W. Weckwerth(1,2), T. Nägele(1,2,3)

(1) University of Vienna, Department of Ecogenomics and Systems Biology, Althanstr. 14, 1090 Vienna, Austria
(2) University of Vienna, Vienna Metabolomics Center (ViMe), Althanstr. 14, 1090 Vienna, Austria
(3) Department Biology I, Ludwig-Maximilians-Universität München, Martinsried, Germany

Under abiotic stress influence, the stabilisation of primary carbohydrate and energy metabolism is essential for plant survival. Stress responses regarding these metabolic pathways have been shown to not only comprise changes in metabolite levels but also contain intricate subcellular translocation and signalling mechanisms [1]. Additionally, futile cycling of sucrose via sucrose cleavage and ATP-consuming hexose phosphorylation makes it difficult to intuitively derive conclusions about its regulation under stress conditions.

In the presented study, a cold susceptible and a cold tolerant natural accession of *Arabidopsis thaliana* were exposed to a combined cold and high light stress revealing significant differences in the dynamics of sucrose and fumarate metabolism of these two accessions. Kinetic modelling of invertase-driven sucrose cleavage revealed differential subcellular invertase reprogramming, pointing to a substantial role of this enzyme in the initial stress response. The cold tolerant accession was shown to rely on sucrose cleavage in the vacuole during stress, while in the cold susceptible accession the cytosolic pathway of sucrose cleavage was more active. Finally, we applied a reverse genetic approach using a mutant being dramatically impaired in vacuolar invertase activity, to approve the central role of this enzyme in stabilizing photochemical processes under freezing and high light conditions [2].

[1] Hörmiller, I., Nägele T., Augustin H., Stutz S., Weckwerth W. & Heyer A. G., "Subcellular reprogramming of metabolism during cold acclimation in Arabidopsis thaliana," Plant. Cell Environ. pp. 1–9, (2016)
[2] Weiszmann, J., Fürtauer, L., Weckwerth, W. & Nägele, T. "Vacuolar invertase activity shapes photosynthetic stress response of Arabidopsis thaliana and stabilizes central energy supply". bioRxiv 168617 (2017)

Analysis of the Role of Galactinol in Abiotic Stress Responses

M. Köhler, R. Tenhaken

University of Salzburg, Dept. of Biosciences

In future, plants have to challenge more and more soil salination, enhanced temperatures and less rainfall due to the ongoing climate change. Under these circumstances the mechanisms behind salt and drought resistance in some varieties are crucial. The UDP-sugar pathway seems to be a determining factor in responding and regulating abiotic stress. Galactinol is a key metabolite in the biosynthesis of raffinose oligosaccharide. It is known that elevated expression of galactinol synthase 2 (GolS2) from *Thellungiella salsuginea* resulted in an increase of galactinol and enhances tolerance against salt stress (Sun *et al. 2002* [1]*).* According to Taji *et al.* (2002 [2]) overexpression of GolS2 in *Arabidopsis thaliana* leads to a significantly higher resistance against drought. The hypothesis that high galactinol levels induce the expression of defense-related genes has to be tested. In order to investigate the influence of galactinol on abiotic stress resistance, *Arabidopsis* lines with significant higher levels of galactinol were generated. Therefore, the effect of different overexpressed genes coding for enzymes of the galactinol pathway, e.g. *myo*-inositolphosphate synthase 1 (MIPS1) or knock out mutants of raffinose synthases (RS), are studied under certain stress conditions.

[1] Sun, Z. *et al.* (2013) Overexpression of TsGOLS2, a galactinol synthase, in Arabidopsis thaliana enhances tolerance to high salinity and osmotic stresses. *Plant Physiol Biochem.* , 69, 82–89.
[2] Taji, T. *et al.* (2002) Important roles of drought- and cold-inducible genes for galactinol synthase in stress tolerance in Arabidopsis thaliana. *Plant J*, 29, 417–426.

Deposition of lignin and suberin into root tip as a reaction to copper exposure and stress

J. Kováč, A. Lux, M. Vaculík

Department of Plant Physiology, Faculty of Natural Sciences, Comenius University in Bratislava, Ilkovičova 6, Mlynská dolina B-2, SK-84215 Bratislava, Slovakia

Various heavy metals induce similar changes in root metabolism and physiology, which can lead to a complex remodeling of root system. Final morphological responses of roots exposed to toxic concentration of heavy metal include root growth inhibition, differentiation of xylem vessels close to the root tip, enhanced suberin lamellae deposition and enhanced lateral root production. Recently, we have found that such changes in root morphology and anatomy are in radish (*Raphanus sativus*) roots coupled with the formation of opaque, non-transparent deposit very close to the root tip. Histochemical analysis of lignin and suberin and analysis of spatial-temporal characteristics was performed. Based on results, we named deposit as a subero-lignified apical deposit (SLAD). This unique structure, not longer than one hundred μm, consists of modified cell walls of central cylinder that are encircled by a short cylinder of prematurely suberized endodermal cells. SLAD starts to form, in both primary and lateral roots, after cessation of root elongation, and it is coupled with xylem differentiation and root branching close to the root apex. Deposition of phenolic substances into SLAD, mainly suberin in endodermis, is spatially separated from suberization or lignification in basally located endodermis. The main reason for formation of SLAD is elusive, we assume that it is a part of stress induced responses which relate to decreased root growth or permeability in heavy metals stress [1].

Acknowledgement: The work was supported by: Slovak Grant Agency VEGA by grant VEGA 1/0605/17; by the Slovak Research and Development Agency under the contract No. APVV SK-AT-2015-0009 and the Scientific & Technological Cooperation (WTZ) Austria & Slovakia SK 04/2016; and by Ernst Mach Grants Action Austria-Slovakia.

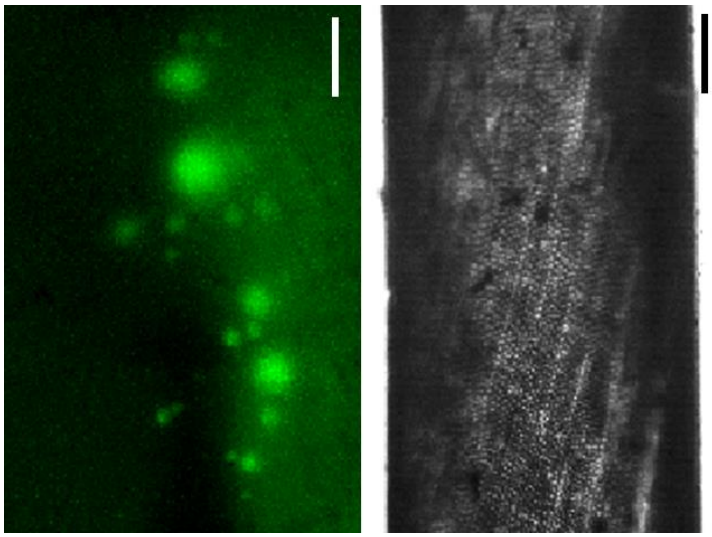
[1] Kováč J., Lux A., Vaculík M., 2018. Annals of Botany, in press

Surface pH changes and salinity response of *Chara australis*

M. Absolonova(1), M.J. Beilby(2), A. Sommer(1), M.C. Hoepflinger(1), I. Foissner(1)

(1) Department of Biosciences, University of Salzburg, Salzburg, Austria
(2) School of Physics, University of New South Wales, Sydney, Australia

When internodal cells of salt sensitive *Chara australis* are exposed to light, conspicuous patterns of acid and alkaline bands are formed at their surface. It has been shown that pH-banding depends on photosynthesis. It is assumed to be caused by inhomogeneous distribution/activation of proton pumps. Electrophysiological measurements suggest that salinity inhibits proton pumps and opens putative H^+/OH^- channels. Based on these findings, we used fluorescein isothiocyanate (FITC) coupled to dextran 70 to monitor pH changes in artificial fresh water and in saline medium. The dextran 70 conjugation prevents the dye to access the cell wall and allows detection in a close proximity of the cell surface. In the early phase of saline stress, alkaline bands vanished and the appearance of transient alkaline spots was observed. After longer saline exposure, some of the spots became fixed in space. The osmotic component of the saline stress neither affected the pH-banding ability nor the transient spot generation [1].



Transient alkaline spots at the surface of a salt-stressed internodal *Chara* cell. (Bar is 100 µm).

[1] Absolonova M, Beilby MJ, Sommer A, Hoepflinger MC, Foissner I (2017) Surface pH changes suggest a role for H^+/OH^- channels in salinity response of Chara australis. Protoplasma.

The role of the endoplasmic reticulum in regulating cytokinin responses

M.C.E. Niemann(1), G. Leonte(1), H. Weber(1), I. Bartrina(2), T. Guo(1), T. Werner(2)

(1) Institute of Biology/Applied Genetics, Dahlem Centre of Plant Sciences (DCPS), Freie Universität Berlin, Albrecht-Thaer-Weg 6, D-14195 Berlin, Germany
(2) Institute of Biolog, Plant Sciences, University of Graz, Schubertstraße 51, 8010 Graz, Austria

Cytokinin is a plant hormone regulating numerous physiological and developmental processes. It is a key morphogenic factor controlling cell division and differentiation, and thereby the activity of plant meristems and organ growth. To govern accurately these different processes, the cellular concentration of cytokinin must be precisely regulated, for instance through the metabolic inactivation mediated by cytokinin oxidase/dehydrogenase (CKX) proteins. Our current research revealed some new mechanisms modulating CKX activity and cytokinin responses.
ROCK1 has been recently identified as an ER-localized facilitator of UDP-GlcNAc and UDP-GalNAc transport in *Arabidopsis*, whose mutation suppresses phenotypes inferred by low cytokinin concentrations. This suppression was caused by the loss of CKX activity *in planta*. *rock1* enhanced the shoot apical meristem activity and organ formation rate, demonstrating an important role of ROCK1 in regulating the cytokinin signal in the meristematic cells through modulating activity of CKX proteins. Biochemical and genetic evidences indicate that the ROCK1 activity is an important part of the ER quality control system eliminating improperly folded proteins from the secretory pathway.
In another line of research, we identified a distinct group of heavy metal-associated isoprenylated plant proteins (HIPPs) as CKX-interacting partners. The physiological function and molecular activity of HIPP proteins is largely unknown. Our molecular and phenotypic analyses of different *HIPP* mutants suggest that the identified genes are involved in different aspects of plant growth, such as root development and leaf formation. Experiments employing a synthetic cytokinin reporter revealed changes in cytokinin status suggesting that the HIPP-CKX interaction is physiologically relevant for cytokinin activities *in planta*. The possible mechanisms underlying the HIPP-CKX interaction are being currently investigated.

Protein modification and plant responses to the environment

A. Bachmair, N. Winter, L. Nehlin

Dept. of Biochemistry and Cell Biology, Max F. Perutz Laboratories, Univ. of Vienna, Dr. Bohr Gasse 9, A-1030 Vienna Austria

The modification of proteins by covalent, frequently reversible, modifications is part of virtually every signal transduction cascade. We are particularly interested in how plants respond to their environment to allow survival and to optimize growth. While changes in gene expression are a final consequence of altered environmental conditions, the immediate response involves modification of existing proteins. The most prominent protein modification is phosphorylation, but the modifier proteins ubiquitin and small ubiquitin-related modifier (SUMO), both of which can be covalently linked to substrates, are also essential for many processes. Moreover, there seems to be an interplay between these modifications, in that different modifications can occur on the same protein, which thereby acts as a hub for signal integration. Our research focuses on two pathways of modification, by SUMO and by ubiquitin. Regarding ubiquitin, we investigate the so-called N-end rule pathway, in which the amino-terminal residue of a protein acts as a signal for ubiquitin addition. This pathway is essential for plants to survive flooding stress [1]. Regarding SUMO, we investigate how chains of SUMO proteins are synthesized, and how generation of SUMO chains influences a plant´s reaction to salt stress [2, 3]. More recently, we also identified (by mass spectroscopy methods) proteins that are modified by both phosphorylation, and by SUMO conjugation [4, 5].
We discuss recent progress in understanding the biochemistry of protein modification. We also present examples how modification signals are integrated into decision making, to regulate stress responses.

[1] Gibbs D.J., Bacardit J., Bachmair A., Holdsworth M.J. (2014) Trends Cell Biol 24, 603-611.
[2] Tomanov K., Zeschmann A., Hermkes R., Eifler K., Ziba I., Grieco M., Novatchkova M., Hofmann K., Hesse H., Bachmair, A. (2014) Plant Cell 26, 4547-4560.
[3] Tomanov K, Nehlin, L., Ziba, I., Bachmair, A. (2018) Biochem J 475, 61-68.
[4] Nukarinen, E., Tomanov, K., Ziba, I., Weckwerth, W., Bachmair, A. (2017) Plant J 91, 505-517.
[5] Tomanov K., Nukarinen E., Vicente J., Mendiondo G.M., Winter N., Nehlin L., Holdsworth M., Teige M., Weckwerth W., Bachmair A. (2018) J Exp Bot, in press.

The cell wall as platform for heavy metal sensing

J. Richter, M.-T. Hauser

Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Muthgasse 18, 1190 Vienna, Austria

Cell walls not only act as a protective barrier surrounding protoplasts but serve as signaling platform between the extracellular environment and the intracellular physiology. Exposure to ions of heavy metals and trace elements leads to changes in the abundance and modification of cell wall components. Our hypothesis is that these metal ion induced cell wall changes are either directly or indirectly sensed by receptor like kinases (RLKs), which mediate growth responses.
In quantitative expression analysis of metallicolous *Salix caprea* genotypes we revealed the induction of genes involved in cell wall metabolism and modification as well as RLKs upon metal treatment [1]. In a molecular genetic approach with the model plant *Arabidopsis thaliana* we further investigated the involvement of selected RLKs in the growth adaption triggered by metal ions. Recently, we have shown for several *Cr*RLK1Ls a role in modulating growth and cell expansion upon copper (Cu), nickel (Ni), zinc (Zn), cadmium (Cd), and lead (Pb) stress [2]. Here we present further experimental evidence and a possible mechanism supporting a model of cell wall receptor mediated metal ion sensing.

[1] Konlechner C., Türktaş M., Langer I., Vaculík M., Wenzel W.W., Puschenreiter M., Hauser M.-T., 2013. Environ. Pollut. 178, 121–127
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The role of O-glycosylation in plant developmental transitions

K.V. Mutanwad, C. Mayrhofer, I. Zangl, D. Lucyshyn

Department of Applied Genetics and Cell Biology, BOKU-University of Natural Resources and Life Sciences Vienna, Austria

Plants undergo several developmental transitions in the course of their life cycle, marked by specific morphological changes such as altered leaf morphology, the formation of trichomes and the onset of flowering. The timing of transition from juvenile to adult phase is regulated by the amount of available sugar accumulating during plant growth. This process is mediated by balancing the ratio between miRNA156 and miRNA172, thus regulating the downstream miRNA156 target genes, a family of SPL (SQUAMOSA PROMOTER BINDING PROTEIN-LIKE) transcription factors. In Arabidopsis, mutants in the protein O-fucosyltransferase SPINDLY (SPY) show accelerated transition from the juvenile to the adult phase, as well as early flowering. O-fucosylation is a posttranslational modification that is closely connected to the O-GlcNAc modification, which is essential for development. In this type of O-glycosylation, either a single fucose or N-acetylglucosamine (GlcNAc) is O-linked to side chains of serine- or threonine residues of nuclear and cytosolic proteins. In the current working model O-fucosylation and O-GlcNAcylation compete for the same targets, with counteracting functional effects. Given the observed early transition phenotypes of *spy*-mutants, we are currently investigating the role of O-glycosylation in the regulation of miRNA156 and miRNA172, potentially in response to the accumulation of sugar in growing tissues. Preliminary data suggest that *spy*-mutants indeed show altered levels of these miRNAs. Moreover, we are testing if O-glycosylation also affects the function of SPLs. These experiments will contribute to our understanding of the molecular mechanisms of O-glycosylation in the regulation of plant development.

Supported by the Austrian Academy of Sciences ÖAW and the Austrian Science Fund FWF.

Arabinokinase, a unique fusion protein involved in arabinose toxicity, is challenging the cellular energy homeostasis kinases

R. Tenhaken(1), E. Kavkova(1), R. Behmüller(1,2), S. Düh(1), C. Huber(2)

(1) University of Salzburg, Dept. of Biosciences, Plant Physiology
(2) University of Salzburg, Dept. of Biosciences, Bioanalytics

Nucleotide sugars are mainly synthesized by de novo pathways from photosynthesis assimilates. The turnover of cell wall polymers or glycoproteins release free monosaccharides, which are recycled into nucleotide sugars. Arabinokinase is a 100 kDa fusion protein of a putative glycosyl-transferase and a sugar-1-kinase domain. This unique gene structure is conserved in all plants down to green algae. The enzyme is localized in the cytoplasm limiting the number of putative substrates. Recycling of L-arabinose requires both, the action of a sugar-specific sugar-1-kinase and a broad substrate accepting UDP-sugar-pyrophosphorylase [1]. These kinases are members of a small superfamily of GHMP-kinases, which have a unique ATP-binding site. Here we characterize a novel kinase for arabinose. A point mutant in this gene, *ara1-1*, is lethal in the presence of L-arabinose, which was previously interpreted as arabinose toxicity [2]. However a knockout in the same gene is tolerated though these mutants accumulate far higher levels of arabinose. Furthermore, D-Ara is not a substrate for arabinokinase and does not cause lethality in *ara1-1*. We will provide data that *ara1-1* is a sugar signaling mutant and requires residual enzyme activity for this action. Target genes of either SnRK1 or Tor-kinase signaling pathways are strongly affected, though glucose and sucrose levels are high [3].

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Significance of hetero-trans-β-glucanase in cell wall metabolism of *Equisetum* and transgenic *Arabidopsis*

K. Herburger(1), L. Franková(1*), A.D. Hudson(1), F. Meulewaeter(2), S.C. Fry(1)

(1) Institute of Molecular Plant Sciences, The University of Edinburgh, Edinburgh, UK; Klaus.Herburger@ed.ac.uk
(2) Bayer CropScience NV, Innovation Center, Gent, BE
**Present address: School of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne, UK*

Transglycanases – cell-wall-localized enzymes occurring in all plants and acting on polysaccharides – cut a donor and reattach it covalently to an acceptor nearby, which is crucial for cell wall and plant development [1]. Hetero-transglycosylation, catalysing bond formation between chemically different polysaccharides, is a little examined phenomenon and the few studies on hetero-transglycanases were conducted *in vitro* [2]. Here we show that recently discovered HTG (hetero-trans-β-glucanase, [3]) from the fern *Equisetum* covalently attaches cellulose to the hemicellulose xyloglucan *in vivo* (cellulose:xyloglucan endotransglucosylase action; CXE). Furthermore, HTG can graft mixed-linkage β-glucan (MLG) onto xyloglucan (MLG:xyloglucan endotransglucosylase action; MXE). Using a novel approach based on radiochemistry and fluorescently labelled xyloglucan molecules combined with selective transglycosylation-targeting inhibitors allowed us to quantify and visualize CXE and MXE action both *in vivo* and *in situ* in various *Equisetum* tissues. Higher HTG specific action in ageing tissues suggests a role of hetero-transglycosylation in wall and tissue strengthening. Introducing the *Equisetum* HTG gene into *Arabidopsis* is currently allowing us to assess ultrastructural changes in the plants and to test for consequences on mechanical properties.

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PGI and 1-FEH activities, a key combination of physiological markers from primary carbohydrate and fructan metabolism to breed high sugar grasses

A. Gasperl(1*), H.W. Pfeifhofer(1), E. van der Graaff(1,2), A. Morvan-Bertrand(3), M.P. Prud'homme(3), M. Müller(1), T. Roitsch(1,4,5)

(1) Institute of Biology, Plant Sciences, University of Graz, 8010 Graz, Austria
(2) Koppert Cress, 2681 MB Monster, The Netherlands
(3) Normandie University, UNICAEN, INRA, EVA, 14000 Caen, France
(4) Global Change Research Centre, Czech Globe AS CR, 664 24 Drásov, Czech Republic
(5) University of Copenhagen, Department of Plant and Environmental Sciences, 1871 Frederiksberg C, Denmark
** Corresponding author: anna.gasperl@uni-graz.at*

High sugar (fructans, sucrose), grasses may provide animal feed with improved nitrogen use efficiency and therefore help to reduce greenhouse gas emissions from farmland [1] and allow the production of non-food crop based biofuels [2]. Perennial ryegrass (*Lolium perenne* L.), is the most important forage grass in Europe. However, regulatory mechanisms of fructan metabolism are poorly understood. Breeders are currently limited to screen candidate populations for water soluble carbohydrate content or few genetic markers, which requires expensive equipment and specific fructan standards or primers often not available for smaller breeding companies.

We present here that recently published enzyme activity platforms [3], [4], are well suited for identifying potential functional markers for the high sugar trait by analysing low and high sugar cultivars of perennial ryegrass. Cold treatment allowed the identification of two novel functional markers positively correlating with the high sugar trait: high or increased phosphoglucose isomerase (PGI) and high or increased fructan 1-exohydrolase (1-FEH) activities. Our analyses further revealed how cultivars maintained high sucrose supply differently under fructan accumulating conditions.

Taken together, these results may prove useful for future breeding strategies and for understanding the regulation of primary carbohydrate and fructan metabolism in perennial ryegrass.

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Funding: This work was supported by a BMBF grant to AG and the WTZ grant FR15/2012 (PHC AMADEUS 2012 number 27206ZE for France) to AG, AM-B, MPP, EVDG and TR and by the Ministry of Education, Youth, and Sports of CR within the National Sustainability Program I (NPU I), grant number LO1415 to TR.

Quantifying the Subcellular Plant Metabolome

L. Fürtauer(1), W. Weckwerth(1,2), T. Nägele(1,2,3)

(1) Department of Ecogenomics and Systems Biology, University of Vienna, Vienna, Austria
(2) Vienna Metabolomics Center, University of Vienna, Vienna, Austria
(3) Department Biology I, Ludwig-Maximilians-Universität München, Martinsried, Germany

Compartmentation is a key feature of eukaryotic cells though biological research is frequently limited by methods allowing for a subcellular resolution of the metabolome. It is discussed and accepted since decades that cellular biochemical research and metabolic regulation can only be resolved if subcellular compartments are taken into account. Technical challenges have frequently limited data reproducibility and sample throughput significantly affecting statistical robustness of subcellular metabolite data. Here, we present a method which is based on the non-aqueous fractionation technique enabling the assignment of metabolites to their subcellular compartment, e.g. vacuole, cytosol or plastid [1]. The method is applicable to resolve subcellular metabolite dynamics in a precise and statistically robust manner. Adapted to a benchtop standard equipment the method is based on the separation of cellular fractions via density gradients consisting of organic solvents. Determination of relative distributions of compartment specific marker enzymes together with metabolite profiles over the density gradient facilitates estimations of compartment specific metabolite concentrations by correlation.
The method was developed, tested and validated in a cold acclimation experiment of three natural accessions of *Arabidopsis thaliana*. Accessions were chosen due to their differential cold acclimation output to unravel different strategies during the metabolic acclimation process. Cold sensitivity was found to particularly correlate with shuffling of amino acids between subcellular compartments. In contrast, cold tolerance was associated with subcellular accumulation of primary metabolites and, hence, we suggest that the intensity of subcellular rearrangements of primary metabolites is negatively correlated with freezing tolerance.
Finally, we conclude that subcellular metabolome analysis is essential to unambiguously unravel different regulatory strategies being involved in plant-environment interactions.

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Control of photosynthesis, glutathione and amino acid metabolism by light quantity and quality in wheat

G. Kocsy(1), D. Toldi(2), M. Gyugos(1), É. Darkó(1), M. Pál(1), K. Gierczik(1,3), G. Galiba(1,3), M. Müller(4), L. Simon-Sarkadi(2)

(1) Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, H-2462 Martonvásár, Brunszvik u. 2., Hungary
(2) Department of Food Chemistry and Nutrition, Szent István University, H-1118 Budapest, Somlói út. 14-16., Hungary
(3) Festetics Doctoral School, Georgikon Faculty, University of Pannonia, Keszthely, Hungary
(4) Institute of Plant Sciences, University of Graz, 8010 Graz, Schubertstraße 51, Austria

Photosynthesis, glutathione and amino acid metabolism are adjusted to changes in the light conditions which is important for the appropriate growth of plants because of their central role in the control of various physiological and biochemical processes. Increase in light intensity was accompanied with a simultaneous increase in the photosynthetic activity and fresh weight of shoots in wheat. Modification of the ratios of blue, red and farred spectral components also affected the photosynthetic electron transport rate which was inhibited by farred light. However, the growth of the seedlings was not influenced by spectrum. Alterations in photosynthesis affected the redox environment since the total glutathione content and the ratio of the glutathione disulphide was greater at higher light intensity compared to the lower ones. In farred light the total glutathione content was smaller compared to the other spectral conditions. The altering availability of reducing power from photosynthesis also influenced the accumulation of free amino acids which was greatly induced by the increase in light intensity and by the blue light. The concentration of most amino acids was much lower in pink light compared to blue light and farred light. However, the lowest contents of proline (Pro) and methionine were observed in blue light. The spectrum had the largest influence on Pro which was shown at both metabolite and gene expression level. Taking into account the role of Pro in the maintenance of the osmotic and redox environment, this amino acid may have a key function in the adjustment of metabolism to the light conditions. The expression of several genes related to redox system and amino acid metabolism increased with increasing light intensity and a few of them was also affected by spectrum. The observed light-dependent alterations in the metabolism of glutathione and free amino acid levels may contribute to the appropriate growth of wheat if the intensity of illumination or its spectral composition changes.

The work is supported by the National Research, Development and Innovation Office (grants ANN 117949, TéT_15-1-2016-0048 and TÉT_15-IN-1-2016-0028) and the EFOP-3.6.3- VEKOP-16- 2017-00008 project. In addition the latter project is co-financed by the European Union and the European Social Fund.

Bridging proteomics, RT-qPCR and microscopy to unravel the spatio-temporal expression changes of hordoindolines across development-dependent changes in barley grains

A. Shabrangy(1#), V. Roustan(1#), S. Reipert(2), M. Weidinger(2), P.-J. Roustan(1), E. Stoeger(3), W. Weckwerth(1), V. Ibl(1*)

(1) Department of Ecogenomics and Systems Biology, University of Vienna, Althanstr. 14, 1090 Vienna, Austria
(2) Core Facility Cell Imaging and Ultrastructure Research, University of Vienna, Althanstr. 14, 1090 Vienna, Austria
(3) Department for Applied Genetics and Cell Biology, Molecular Plant Physiology and Crop Biotechnology, University of Natural Resources and Life Sciences, Muthgasse 18, 1190 Vienna, Austria
#A. Shabrangy and V. Roustan have contributed equally to this work
**Correspondence: Dr. V. Ibl, Department of Ecogenomics and Systems Biology, University of Vienna, Althanstr. 14, 1090 Vienna, Austria, verena.ibl@univie.ac.at , Twitter: @VerenaIbl*

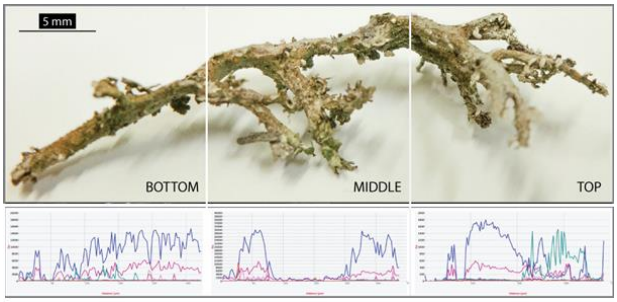
Grain hardness is one of the quality parameters of cereal grains. Hordeum vulgare (barley) hordoindolines (HINs), HINa, HINb1 and HINb2, are orthologous proteins of wheat puroindolines (PINs) that are small, basic, cysteine-rich seed-specific proteins and responsible for grain hardness.
In barley, HINb is explicitly and mostly expressed at mid-stage developed endosperm and is associated to both major endosperm texture and grain hardness. However, data are still missing to understand the dynamics of HIN transcripts and protein regulation during grain filling processes.
Bridging qRT-PCR, proteomics and microscopy we analyzed RNA transcripts and protein abundance from the whole seeds at 4 developmental stages as well as from aleurone, subaleurone and starchy endosperm at 12 and 20 days after pollination (dap) by laser microdissection (LMD). In this context, reference genes were validated and reference proteins were identified for the barley cultivar Golden Promise to measure the relative abundance of HIN RNA transcripts and proteins during barley endosperm development for the whole seed as well as for the spatio-temporal samples. At the whole seed level, results from qRT-PCR, proteomics and western blot show a continuous increase of HIN RNA transcripts and protein abundance across the developmental stages. Localization studies reveal HIN localization at the vacuolar membrane in the aleurone, at protein bodies (PBs) in subaleurone and at the surface of starch granules in the starchy endosperm.
Quantification analyses identified HINb2 as the most prominent protein in starchy endosperm at 12 and 20 dap. Additionally, both LMD proteomics and RNA transcript data suggest that HINa protein preferentially accumulate at 20 dap in subaleurone.
Here, we present for the first time a spatio-temporal overview of both HIN RNA transcripts and protein abundance in developing barley grain. Our data indicate a contribution of each tissue to the expression of HINs during grain filling will help to understand the molecular mechanism behind these tissue and stage- dependent rearrangements and will subsequently improve cereal quality traits.

Localization and identification of calcium oxalate forms in lichens

M. Goga(1,2), D. Ručová(1), M. Marcinčinová(1), M. Baláž(3), M. Weidinger(2), I. Lang (2)

(1) Department of Botany, Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafárik University, Mánesova 23, 041 67, Košice, Slovakia
(2) Core Facility Cell Imaging and Ultrastructure Research, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria
(3) Institute of Geotechnics, Slovak Academy of Sciences, Watsonova 45, 040 01 Košice, Slovakia

Lichens represent a unique form of symbiosis between photosynthetic and fungal partners. They belong to one of the most sensitive organisms at the level of ecosystem pollution. Atmosphere deposition is the major source of mineral nutrition for lichens [1] but in some specific situations, the substrate plays also an important role in the accumulation of mineral elements, including essential metals like calcium [2]. Calcium is important for structures in cell walls as well as in biological membranes and it is an essential intracellular messenger in the cytosol [3]. Oxalic acid is usually associated with calcium in lichens where it leads to the formation of calcium oxalate crystals. There are two forms of calcium oxalates in lichens: weddelite and whewellite [4]. However, the localization of calcium and the identification of oxalate forms in lichens is largely unknown.
In a survey of travertine localities (calcareous soils) in Slovakia, we found two lichen species *Cladonia foliacea* and *Cladonia furcata* (*cladoniaceae*). Energy-dispersive X-ray spectroscopy (EDX) revealed calcium uptake from the soil and its localization in the thalli of both species. Using a diffractometer and according to JCPDS-PDF2 database [5], we can distinguish two calcium oxalate forms. This localization and accumulation of calcium oxalate in lichen thalli is an essential contribution to better understand calcium distribution and function in lichens.



Lichen thallus of *Cladonia furcata* and linescan of elements by EDX.

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Stress signaling networks in algae and plants

W. Weckwerth

Department of Ecogenomics and Systems Biology, University of Vienna
Vienna Metabolomics Center (VIME), University of Vienna
Email wolfram.weckwerth@univie.ac.at

Stress perception in algae and plants is transduced by highly complex signaling networks. These signaling networks have direct consequences on cellular processes and overall physiology of the algae and plant. Recently, we have investigated the SnRK1 dependent signaling network in *Arabidopsis thaliana* and revealed an intimate connection with TOR signaling (1). These pathways seem also to be interwoven in nitrogen starvation and recovery experiments in *Chlamdomonas reinhardtii* as we demonstrated in recent studies where we integrated physiological with metabolomics, proteomics and phosphoproteomics data (2, 3). The antagonistic AMPK-TOR signaling pathways are highly conserved from animals to plants (4). I will review our studies on these signaling pathways and further discuss consequences for plant and algae stress physiology.

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Ionic Stress induces Fusion of Mitochondria to 3-D Networks: an Electron Tomography Study

P. Steiner(1), M. Luckner(2), H. Kerschbaum(1), G. Wanner(2), U. Lütz-Meindl(1)

(1) Department of Biosciences, University of Salzburg, Hellbrunnerstraße 34, A-5020 Salzburg, Austria
(2) Ultrastructural Research, Faculty of Biology, Ludwig-Maximilians-University, Großhadernerstraße 2-4, D-82152 Planegg-Martinsried, Munich, Germany

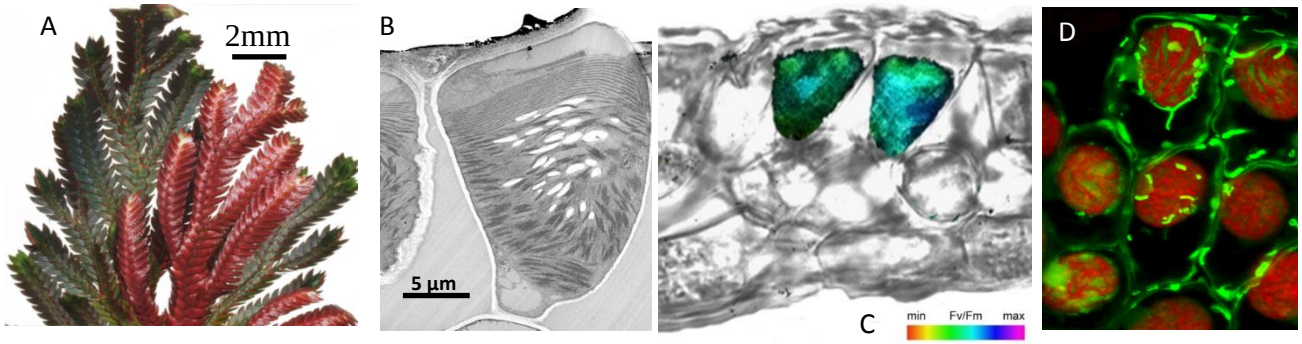
Mitochondria are central organelles for energy supply of cells and play an important role in maintenance of cytoplasmic ionic balance. Consequently mitochondria are highly sensitive to any kind of stress to which they mainly response by disturbance of respiration, ROS production and release of cytochrome c into the cytoplasm. Physiological and molecular reactions of mitochondria to stress are well known, yet there is a lack of information on stress induced structural changes that can be regarded as stress hallmarks. 3-D reconstructions of high-pressure frozen cells by FIB-SEM- and TEM-tomography provide an excellent tool for studying mitochondrial structural stress reactions. In the present study it is shown that mitochondria in the unicellular algal model system *Micrasterias* as well as in the closely related aquatic higher plant *Lemna* fuse to local networks as consequence of ionic stress. In dependence on concentration and duration of the treatment, fusion of mitochondria occurs either by formation of protuberances arising from the outer mitochondrial membrane, or by direct contact of the surface of elongated mitochondria. As our results show that respiration is maintained in both model systems during ionic stress and mitochondrial fusion, as well as formation of protuberances are reversible, we assume that mitochondrial fusion is a ubiquitous process that may help the cells to cope with stress.

Structure and physiology of giant chloroplasts in the deep shade adapted lycopod *Selaginella erythropus*

R. Ghaffar(1,3), M. Weidinger(1), B. Mähnert(2), M. Schagerl(2), I. Lichtscheidl(1)

(1) Core Facility Cell Imaging and Ultrastructure Research, University of Vienna, Austria
(2) Department of Limnology and Bio-Oceanography, University of Vienna, Austria
(3) Department of Botany, University of the Punjab, Lahore, Pakistan

Chloroplasts of deep shade plants are models for the investigation of plant adaptation to different light conditions. We analysed giant chloroplasts of *Selaginella erythropus*, a lycopod from the extremely dark undercover of rain forests [1]. They are characterized by a specific organization of their thylakoids into (a) a zone which is rich in grana stacks thus resembling typical chloroplasts of higher plants, and (b) a cover of parallel thylakoids on top reaching over the whole plastid [2].
We describe the rearrangement of thylakoid membranes in these bizonoplasts reflecting their structural flexibility towards natural conditions of high light (sun flecks) or extensive shading. An interesting observation was the *de novo* formation of transitory prolamellar bodies in fully mature bizonoplasts during prolonged periods of darkness.
The structural response was correlated with physiologic experiments about pigment contents and photosynthetic activity as well as the formation of reactive oxygen species [3].



Microphylls of the lycopod *Selaginella erythropus* (A) showing intact bizonoplasts under weak illumination in the transmission electron microscope (B) and during investigation with micro-PAM (C). Upper zones with long extended parallel thylakoid stacks show weaker variable fluorescence compared to lower zones with typical grana stacks and stroma lamellae. Reactive oxygen species yield green fluorescence with the dye H2DCF.

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Acknowledgements:
We are indebted to our gardeners Andreas Schröfl & Thomas Joch (Dept. Eco-genomics & Systems Biology) for taking care of the plants. This work was funded in parts by Hochschuljubiläumsstiftung der Stadt Wien (H-2115/2010) and Higher Education Commission Pakistan (HEC) to R. Ghaffar.

The cortical endoplasmic reticulum before and after plasmolysis in *Physcomitrella patens*

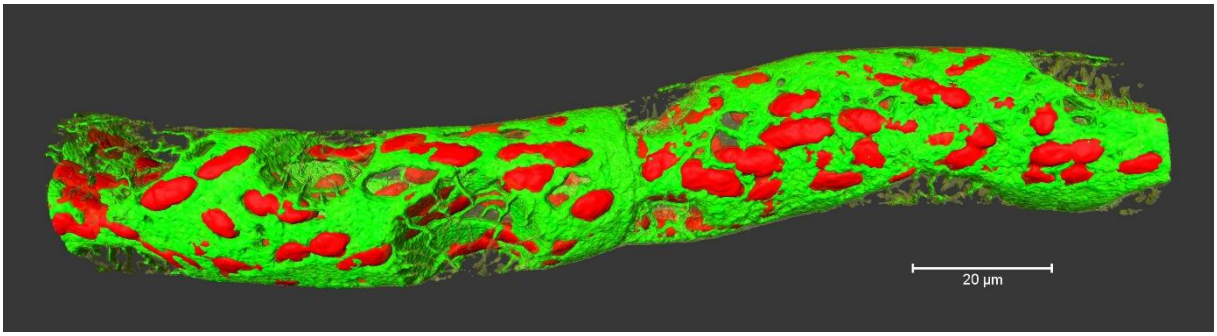
D. Harant, I. Lang

Cell Imaging and Ultrastructure Research, Faculty of Life Sciences, The University of Vienna

The endoplasmic reticulum (ER) is a vital cell organelle in the cortex of eukaryotic plant cells. It plays a decisive role in the synthesis and quality control of protein production [1]. Additionally, it connects the nucleus, the Golgi apparatus and various organelles like chloroplasts and mitochondria and also provides the connection between neighboring cells through plasmodesmata [2].

Here, we examined the cortical ER in protonema cells of a transgenetic *Physcomitrella patens* line (GFP:HDEL). The plasma membrane was labelled simultaneously with fluorescent dye FM4-64 to achieve various insights by confocal laser scanning microscopy (CLSM). By placing the protonemata in a hypertonic Mannitol solution we were able to follow the behavior of the cortical ER and the protoplast during plasmolysis.

In its natural state, the cortical ER is a dynamic network of fine tubes and cisternae underneath the plasma membrane. Under acute and long term-plasmolysis changes in protoplast form and the cortical ER as well as the formation of Hechtian strands and Hechtian reticula [3] were observed. Furthermore, we defined categories for protoplast detachments between two neighbor cells and accomplished a quantitative analysis of their frequency. Processing of the high resolution z-scans allowed the creation of 3D models and gave detailed insights into living protonema cells before and after plasmolysis, in particular their connecting cell walls.



3D-model of two neighboring protonema cells of *P. patens* after plasmolysis in 0.8 M Mannitol.

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Dissecting the effectome of the maize pathogen *Ustilago maydis*

F. Navarrete, S. Uhse, J. Bindics, A. Djamei

Gregor Mendel Institute (GMI), Austrian Academy of Sciences (OEAW), Vienna BioCenter (VBC), Dr. Bohr-Gasse 3, 1030 Vienna

Biotrophic plant pathogenic fungi employ a battery of small secreted molecules, so called effectors, to suppress host defense responses and to redirect the host metabolism in favor of the invader. Although effector proteins are shaping the interaction between the pathogen and the host, their specific function stay often elusive as they largely show no sequence homology to proteins with known functional domains. The smut fungus *Ustilago maydis* causes galls on all aerial parts of the host plant maize. This basidiomycete became in the past decade a model to study biotrophic plant fungal interactions.
In a systematic approach we functionally elucidate the effectome comprising several hundred putative small secreted proteins of *Ustilago maydis,* to learn more about specific effector functions and their plant target proteins.
Here I will describe our progress in identifying specific effector functions and the molecular analysis of the respective plant sided host targets.

Selective modification of the wheat seed microbiota affects hydrogen peroxide production in wheat seedlings

D. Gerna(1), T. Roach(1), W. Stöggl(1), B. Mitter(2), I. Kranner(1)

(1) Department of Botany, University of Innsbruck, Austria
(2) Department of Bioresources, Austrian Institute of Technology (AIT), Austria

Seed performance underpins agricultural productivity and food security. Despite the emerging number of reports on the seed microbiome [1], the contribution of bacterial endophytes to seed germination and vigour remains unclear. We investigated seed-microbe interactions in bread wheat (*Triticum aestivum* L.). Seed surface sterilisation with a "hot steam" treatment significantly reduced the number of microbes, accelerated germination, and decreased rates of hydrogen peroxide (H_2O_2) production, compared to non-surface-sterilised seeds, used as control. Considering the production of H_2O_2, which is a key signalling compound in biotic stress response [2,3], we tested whether seed inoculation with single bacterial strains were associated to elevated levels of H_2O_2 production by wheat seedlings. The microbiota of dry wheat seeds was dominated by *Gammaproteobacteria*, in the families of *Pseudomonadaceae* and *En*terobacteriaceae, as revealed by 16S rRNA gene sequencing. Typical for wheat seeds, the genus *Pantoea*, which is known to infect different crops and induce systemic acquired resistance [4], abounded. Restriction fragment length polymorphism and sequencing of the intergenic spacer region, combined with sequencing of the *gyrase B* gene, identified two species of *Pantoea* (spp. *agglomerans* and *eucalypti*). Interestingly, inoculating surface-sterilised seeds with *P. agglomerans* and *eucalypti* stimulated wheat seedlings to increase and decrease H_2O_2 production, respectively, after 48 h from the onset of imbibition. An H_2O_2 burst was also measured in seedlings exposed to pure cultures of *P. agglomerans* and eucalypti. We then hypothesised that the differential responses of wheat seedlings to distinct *Pantoea* spp. were related to successful endophytic colonisation and different species lifestyles. Therefore, 48 h after the onset of imbibition, endophytic and epiphytic isolates from wheat seedlings were sequenced to study whether selective inoculations of bacterial isolates in dry surface-sterilised seeds alter the microbiota composition, and whether this affected wheat physiology, during seed germination and seedling growth. Our overall aim is to improve the understanding of plant-bacterial interactions in seeds and seedlings, and decipher how the seed microbiota can be optimised to enhance seed germination and vigour.

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Marker-assisted selection for Tomato Mosaic Virus resistance in local varieties of *Solanum lycopersicum* L.

A. Jammer, D. Illmeier, M. Müller

Institute of Biology, Plant Sciences, University of Graz, Schubertstraße 51, 8010 Graz, Austria

The increasing demand for traditional and local tomato varieties raises a new challenge for tomato growers and plant breeders: Certain pathogenic viruses re-emerge that play only a minor role in commercial tomato production due to the use of cultivars bred for resistance. Local varieties mostly lack resistance traits and are, thus, more prone to virus-induced yield losses. Additionally, non-resistant host varieties provide an environment in which viruses may propagate and/or recombine and produce novel genotypes that might be able to break the resistance alleles present in widely used high-performance cultivars [1].

In modern tomato cultivars, the *Tm-1*, *Tm-2* and *Tm-2a* alleles introgressed from wild tomato species confer resistance against the majority of known strains of Tomato Mosaic Virus (ToMV) [2]. In co-operation with ARCHE NOAH [3], an organization that aims to preserve and develop the diversity of cultivated plants, we established two methods for marker-assisted selection (MAS) for the presence of the *Tm-2a* resistance allele in the genome of local tomato varieties. Both the CAPS (Cleaved Amplified Polymorphic Sequences) markers [4] and the tetra-primer ARMS (Amplification-Refractory Mutation System) PCR markers [2] proved to be robustly applicable for the detection of *Tm-2a* in all of the tomato varieties they were tested with. The susceptible and resistant phenotypes observed in a ToMV inoculation experiment coincided with the expectations according to the results of the genotyping of the plants, and the heterozygous presence of the *Tm-2a* allele was sufficient to confer ToMV resistance.

Based on the presented findings, MAS can be applied in future breeding approaches to introduce *Tm-2a*-mediated ToMV resistance into traditional and local varieties, which will contribute to limiting the future spread of ToMV.

The authors would like to thank P. Lammer (ARCHE NOAH e.V.) for initiating the co-operation and providing tomato seeds of local cultivars for experiments, S. García-Martínez (Universidad Miguel Hernández, Elche, Spain) for providing seeds of tomato lines homozygous for *Tm-2a*, and S. Grausgruber-Gröger (AGES GmbH [5]) for providing greenhouse space for the ToMV inoculation experiment and for supporting us with the ToMV inoculation and with the evaluation of symptoms.

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Interactive effects of biotic and abiotic stress factors during seed production on *Helianthus annuus* seed quality

E. Arc(1), E. Castillo-Lorenzo(2), W. Stöggl(1), S. Aigner(1), C. Seal(2), H.W. Pritchard(2), C. Bailly3), I. Kranner(1)

(1) Department of Botany and Center for Molecular Biosciences Innsbruck, University of Innsbruck, Sternwartestraße 15, 6020 Innsbruck, Austria
(2) Royal Botanic Gardens, Kew, Ardingly, West Sussex RH17 6TN, United Kingdom
(3) Developmental Biology Laboratory, Pierre and Marie Curie University, 7 Quai Saint Bernard, Paris, France

Seed quality is of paramount importance to agriculture, food security and the conservation of wild species. This trait is strongly influenced by the environmental stresses experienced by the mother plant. Within the framework of the FP7-project "EcoSeed", a consortium of 11 partners attempted to unravel possible consequences of predicted climate change scenarios on seed quality. In addition to laboratory experiments under controlled conditions, field trials were conducted in collaboration with a seed breeding company. *Helianthus annuus* was chosen as a model oil crop for the field study. Hence, sunflower seeds were produced under either standard conditions or mild drought stress, induced by discontinuing plant irrigation during seed maturation. Approximately half of the seeds were infected by a fungus, *Rhizopus oryzae*, resulting in clear lesions of cotyledons. Infected and non-infected seeds were separated before assessing seed physiological and biochemical traits. The metabolite composition of embryonic axes and cotyledons isolated from infected or non-infected seeds was investigated by GC-MS-based metabolite profiling and targeted analysis of plant hormones. Our study revealed both additive and interactive effects of the biotic (fungi infection) and abiotic (drought) stress factors experienced by the mother plant on the quality of the produced seeds.

Acknowledgments: This work was supported by the European Union (FP7 grant 311840 “EcoSeed”).

Complex patterns in xylem hydraulics of subalpine dwarf shrubs

A. Ganthaler, S. Mayr

Department of Botany, University of Innsbruck, Sternwartestraße 15, 6020 Innsbruck
andrea.ganthaler@uibk.ac.at

Dwarf shrubs are an essential component of high mountain ecosystems in the European Alps. They form extensive dwarf shrub communities in the subalpine and alpine vegetation belt and are adapted to the extreme environmental conditions in high-alpine habitats. Respective adaptations can also be expected regarding hydraulic traits. However, little is known about the hydraulic architecture of this growth form so far.

We analysed the safety and efficiency of water transport in six wide-spread dwarf shrub species of the European Alps (*Vaccinium myrtillus, V. gaultherioides, Calluna vulgaris, Arctostaphylos uva-ursi, Kalmia procumbens* and *Erica carnea*) on various sites in Tyrol, Austria. Hydraulic conductance and vulnerability to drought-induced embolism were investigated by flowmeter measurements, staining with safranine, and non-destructive micro-CT observations.

Measurements revealed that the analysed dwarf shrubs show a surprisingly high proportion of functionally non-active xylem conduits, without being supposed to water stress. Dysfunctions were partly reversible or non-reversible, consistent during the growing season, and assignable to two characteristic distribution patterns within the cross section. Non-functional xylem elements dramatically reduced the potential hydraulic conductivity by 19 to 49%. Water potential at 50% loss of conductivity varied between -1.8 and -2.9 MPa between species.

We present possible explanations for this phenomenon and suggest that xylem function is definitely more dynamic than presumed. High transport capacities may be of minor importance for dwarf shrubs due to their low growth height, and the relatively low hydraulic safety may be compensated by an extensive root system, fast formation of new sprouts after drought events and efficient refilling of embolised conduits. Ecological as well as methodological implications of the hydraulic characteristics found are discussed.

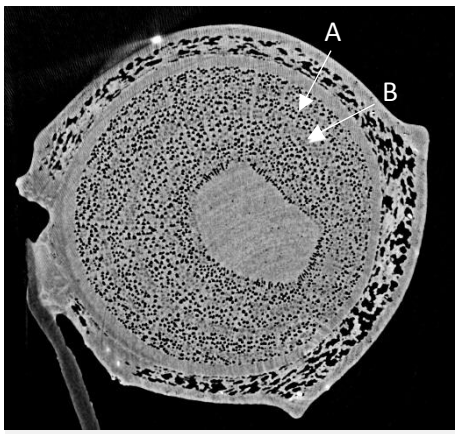

Figure 1. Micro-CT scan showing the stem cross section of *Vaccinium myrtillus*. Non-functional (air-filled) conduits appear deep black (A), while functional (water-filled) conduits appear dark grey (B).

We acknowledge financial support by the Austrian Science Fund (FWF) P29896-B22 and the L'Oreal fellowship 'For Women in Science'. Micro-CT scans were performed in collaboration with the Elettra Synchrotrone Trieste.

Long-term trends in leaf level gas exchange mirror tree-ring derived intrinsic water-use efficiency of *Pinus cembra* at treeline during the last century

G. Wieser(1), W. Oberhuber(2), B. Waldboth(2), A. Gruber(2), R. Matyssek(3), R.T.W. Siegwolf(4), T.E.E. Grams(3)

(1) Department of Alpine Timberline Ecophysiology, Federal Research and Training Centre for Forests, Natural Hazards and Landscape (BFW), Rennweg 1, 6020 Innsbruck, Austria;
(2) Institute of Botany, Leopold-Franzens-Universität Innsbruck, Sternwartestraße 15, 6020 Innsbruck, Austria;
(3) Ecophysiology of Plants, Department of Ecology and Ecosystem Management, Technische Universität München, Von-Carlowitz-Platz 2, 85354 Freising, Germany,
(4) Paul Scherrer Institute, 5232 Villigen, Switzerland.

The ability of treeline conifers in the Central European Alps to cope with recent climate warming and increasing CO_2 concentration is still poorly understood. We determined basal area increment (BAI) and tree ring stable carbon isotope ratios ($\delta^{13}C$) of *Pinus cembra* trees from 1925 through 2013. Stable isotope ratios and BAI were compared with leaf level gas exchange measurements carried out *in situ* between 1934 and 2012, and thus, provided new insights into long-term trends of tree-ring derived intrinsic water-use efficiency (iWUE). Mature *P. cembra* trees at treeline responded to increasing C_a and air temperature with a parallel increase in maximum net CO_2 uptake rate at ambient CO_2 (A_{max}) and tree-ring-derived intercellular CO_2 partial pressure (C_i). A_{max} tripled and was positively correlated to BAI and C_i. The latter increased in parallel with ambient CO_2 concentration and stomatal conductance. In contrast to the instantaneous gas exchange parameters, $\delta^{13}C$ derived iWUE informs about the long-term changes in the carbon water relations. These data showed three changes in the iWUE chronosequences, which could be identified with different long term gas exchange patterns: (1) from stomatal controlled functioning from 1925 to 1981, to a situation where (2) both net CO_2 fixation (A) and leaf conductance for water vapour (g_w), responded to the environment from 1982 to 1997, and (3) back to a stomata controlled pattern over iWUE from 1998 onwards. This temporal pattern was also mirrored in leave level gas exchange assessments, suggesting a parallel increase of A and g_w of *P. cembra* at treeline during the last nine decades.

Posters

(in alphabetical order)

Lignification of endocarp cells during the development of the walnut fruit

S.J. Antreich, N. Xiao, N. Gierlinger

Institute for Biophysics, Department of Nanobiotechnology, University of Natural Resources and Life Sciences, Muthgasse 11, 1190 Vienna

The pericarp of the walnut fruit undergoes a metamorphosis from a soft envelope to a hard shell. During ripening the exo- and mesocarp of the drupe stays green and soft but the endocarp solidifies to a hard shell surrounding the seed. This development starts several weeks after the catkins appearance and ends with the fruit ripening. To deepen our understanding how nature builds up hard packaging structures, walnuts were collected from June to October to study the development of the endocarp.
First of all an overview of the cross-section of the walnut was photographed monthly at each developmental stage, followed by cryo- or rotary microtomy to get microsections (8µm) for Raman, bight-field, and scanning electron microscopy (SEM) investigations.
The overview of the walnuts revealed an increase of the circumference of the endocarp only from June to July, but no further increase in the other months. From July on, the endocarp can be separated into a dark-brown and a light-brown layer, where the dark-brown layer more and more replaced the light-brown layer until October.
Microscopic pictures revealed, that the amount of cell wall filled cells in the endocarp increases during the development. In June the cells of the endocarp showed no thickening of the cell walls. The cell wall thickness increased in July, starting first at the outer most cell layers. In August the cells closest to the outside were completely filled by the cell wall. The width of this layer expanded in September and October further to the cavity.
Micrographs from the SEM analysis showed the difference in the cell wall thickness in July and in August. In July the cell wall was composed by several layers, however, the plasmodesmata were still open. Contrary to that, the cells in August were filled by the cell wall and the plasmodesmata showed some kind of filling.
Raman imaging of the sections showed that the cell wall gets lignified. This lignification of the cells already occurs in July and mainly is completed in August. This early development of the endocarp matches with the strong nutrient uptake found by Drossopoulos et al. in *Juglans regia* in Greek, where the nutrient demand of the endocarp increases rapidly in the first two month after lignification starts [1]. From August on, the cells of the outside of the endocarp are already filled with the cell wall and the lignification process moves further to the inside of the endocarp. Further, when the lignification of the cells is completed, the plasmodesmata of these cells are sealed and the cells dry out. This can be seen as colour changes of the dark-brown layer of the endocarp, which starts in September at the outside of the endocarp. With the fruit fall in October the walnut seed is encapsulated in an impermeable, dry and hard packaging structure.

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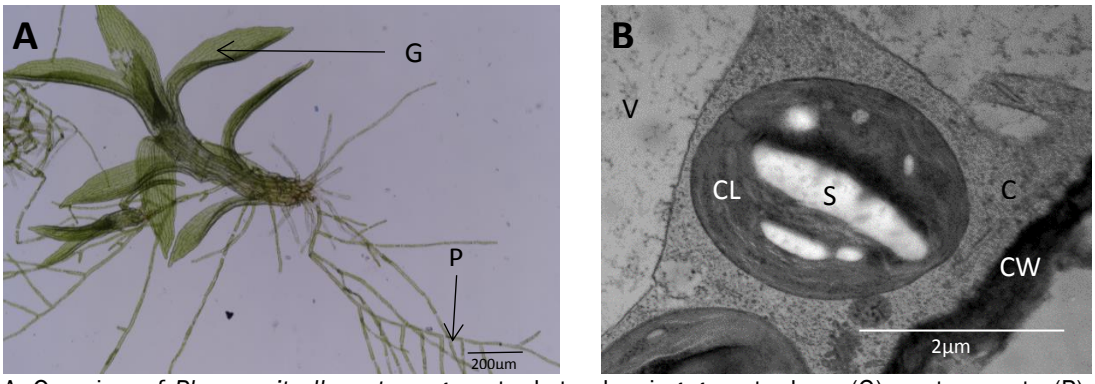
“This project has received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation programme (grant agreement No [681885])”.

A fast cryo-preservation technique of *Physcomitrella patens* for element analysis in the electron microscope

M. Eckhard, S. Reipert, I. Lang

University of Vienna, Core Facility Cell Imaging and Ultrastructure Research (CIUS), Althanstraße 14 1090 Vienna
margret.eckhard@univie.ac.at

In my master thesis, I'm establishing a protocol for the best preservation method for electron microscopy with a special focus on energy loss spectroscopy (EELS). The final aim is the quantification and localization of the heavy metal copper in moss cells of *Physcomitrella patens*.
Physcomitrella patens is a perfect study object for my research because it is a well-known model organism [1] and easy to cultivate under laboratory conditions. Furthermore, it has been shown to be tolerant to heavy metals [2]. The thick cell wall of the moss cells is a challenge in the preparation for the transmission electron microscope (TEM).
To achieve my goal, the combination of light and electron microscopy techniques were necessary. On the electron microscopy level, I compare protocols for chemical fixation and cryofixation. The cryofixation was performed with the HPM 100 (Leica microsystems) and the following freeze substitution was completed with the AFS 2 (Leica microsystems) in combination with a new agitation module [3].
By now, the best fixation results were established by using high-pressure freezing combined with rapid freeze substitution. Previous light microscopic observations showed very diverse reactions of the gametophore and the protonemata of *Physcomitrella patens* to the provided fixation buffers. Therefore, it is necessary to adjust the buffer depending on the part of the moss that should be preserved and to adapt the chemical fixation protocol.
With the combination of all the techniques, I was able to detect the copper in the moss cells.



A: Overview of *Physcomitrella patens* gametophyte showing gametophore (G), protonemata (P); B: TEM of gametophore leaf, cryofixed and freeze substituted. vacuole (V), chloroplast (CL), starch (S), cytoplasm (C), cell wall (CW).

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In vivo and *in vitro* characterization of monolignol oxidoreductases from the berberine bridge enzyme-like protein family in *Arabidopsis thaliana*

R.S. Eggers(1), B. Daniel(1), B. Konrad(1), J. Messenlehner(1), M. Toplak(1), S. Wallner(1), A. Pichler(1), M. Müller(2), A. Jammer(1,2), P. Macheroux(1)

(1) Institute of Biochemistry, Graz University of Technology, Petersgasse 12/2, 8010 Graz, Austria
(2) Institute of Biology, Plant Sciences, University of Graz, Schubertstraße 51, 8010 Graz, Austria

Flavoproteins are a diverse protein class employing an isoalloxazine ring for catalysis in form of the flavin mononucleotide (FMN) or the flavin adenine dinucleotide (FAD). Among them is the berberine bridge enzyme-like (BBE-like) protein family (pfam 08031) named after the berberine bridge enzyme (EcBBE) from California poppy (*Eschscholzia californica*). BBE-like proteins form a multigene family in plants and the number of members varies from one in the moss *Physcomitrella patens* to 57 in the western poplar (*Populus trichocarpa*). Despite the frequent occurrence of these proteins, their function is largely unknown. Therefore, we chose to investigate the genes coding for BBE-like proteins from *Arabidopsis thaliana* to broaden our understanding of this protein family. Here we present the *in vitro* characterization of AtBBE-like protein 13, 15 and 26 and the *in vivo* characterization of putative loss-of-function mutants of the respective genes.
AtBBE-like proteins 15 and 13 were expressed heterologously and identified as monolignol oxidoreductases. They oxidize the monolignols, major cell wall components, to the corresponding aldehydes. This suggests a role of BBE-like proteins in monolignol metabolism and lignin formation that was prior not recognized for this protein family. The structure of AtBBE-like protein 15 was elucidated by protein crystallography. The catalytic machinery can be divided into the active site and a substrate-binding site. 14 out of 28 AtBBE-like proteins share the same active site, but only AtBBE-like protein 26 was found to additionally share the same substrate-binding site as AtBBE-like protein 15 and was therefore classified as a monolignol oxidoreductase.
To complement our understanding of the BBE-like protein family we have initiated a multidimensional approach on the characterization of AtBBE-like proteins 13, 15 and 26 *in planta*. This includes the characterization of putative loss of function mutants and the generation of lines for histochemical staining using the GUS reporter system as well as GFP constructs for subcellular localization. Further, we are also interested in the impact of the respective proteins on the lignin polymer. Therefore, we aim for the analysis of the lignin content and lignin composition of loss-of-function mutants and their soluble phenolics by metabolomic fingerprinting.

Constitutive Endocytosis in *Chara* Internodal Cells: Comparison between Plasma Membrane Dyes and Fluid Phase Markers

A. Sommer, M. Hoeftberger, I. Foissner

University of Salzburg, Department of Biosciences, Hellbrunnerstr. 34, 5020 Salzburg, Austria

Eukaryotic cells internalize plasma membrane and extracellular material by endocytosis. Endocytosis is required for the recycling of plasma membrane components, for nutrient uptake and for signaling. It can be classified according to the cargo (e. g. fluid-phase endocytosis) or according to the mechanism of endocytosis which is used to form an endocytic vesicle from the plasma membrane (e.g. clathrin-mediated endocytosis). The process of endocytosis can be followed *in vivo* with the aid of fluorescent dyes. Among them, FM styryl dyes are most frequently used. They incorporate into the plasma membrane and are taken up via endocytic vesicles which fuse with other organelles thereby revealing the endocytic pathway. In the present study we compared the internalization of these membrane markers with the uptake of Alexa 488 hydrazide, a fluid phase marker, in characean internodal cells. Both dyes were actively taken up into the cytoplasm and stained various classes of endosomes, including brefeldin A- and wortmannin-sensitive organelles (trans Golgi network and multivesicular endosomes). Uptake of FM-dyes as well as of Alexa 488 hydrazide was independent of an intact actin cytoskeleton but could be inhibited by ikarugamycin and methyl ß-cyclodextrin indicating the involvement of clathrin and sterols, respectively. In spite of these similarities, membrane endocytosis markers and fluid-phase markers co-localized only partially. Interestingly, both markers distributed not only to rapidly recycling compartments but were also present in long-lived endosomes. The significance of these findings for the mechanism of constitutive endocytosis in *Chara* internodal cells and for the use of Alexa 488 hydrazide as endocytic marker is discussed.

Transcript and phytohormone analysis based prediction of genotype-specific biological processes in cold-treated wheat

B. Kalapos(1,2), F. Marincs(3), G. Galiba(1,2)

(1) Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Martonvásár, Hungary
(2) Festetics Doctoral School, Georgikon Faculty, University of Pannonia, Keszthely, Hungary
(3) Agricultural Biotechnology Institute, National Agricultural Research and Innovation Centre, Gödöllő, Hungary

Agriculture is highly exposed to climate change that is one of the most critical factors in the crop production nowadays. The average number of extreme weather events per year has increased dramatically in the last 30 years. The temperature fluctuations, as well as low temperature, are greatly able to suppress the development of plants. In this study we focused on the effect of cold shock on gene expression and the level of different hormones in freezing-tolerant and freezing-sensitive genotypes. The selection of plant materials was based on their growth habit and the level of tolerance against frost. Therefore, one genotype with winter growth habit and high frost tolerance [Cheyenne], one with spring growth habit and medium sensitivity [Chinese Spring] and one chromosome substitution line with spring growth habit and frost tolerance [CS(Ch5A)] were chosen. The plants were grown under normal conditions, thereafter they were treated with 4 °C for a day. The tissue samples were taken from leaves, after that they were used for hormone metabolite and gene expression measurements with microarray and qRT-PCR. For data analysis and processing, mainly on-line bioinformatics databases and tools were applied. The level of phytohormones (cytokinins, auxins, abscisic acid, salicylic acid, jasmonates) and their metabolites were determined in all examined wheat genotypes under the effects of short term cold stress. Cluster analysis was performed whose results showed the clear separation of Chinese Spring variety from the other two genotypes including Cheyenne and the substitution line as well. Network analysis based tools were applied to reveal the connections between the Ca-signalling, kinases, receptor kinases, wall-associated receptor kinases, ABA-signalling, transcription and translation related genes. Finally, it should be emphasized that the identified, differentially regulated genes, beyond the above discussed general relationships with cold stress response, can have a more concrete role in the development of freezing-tolerant wheat genotypes. Based on the results above, a number of genes with the potential to increase cold tolerance in wheat might be found amongst the genes that we identified in our study.

This research work was supported by the Hungarian Scientific Research Fund (OTKA CNK80781, OTKA K111879) and by the Hungarian National Development Agency (TÁMOP-4.2.2.B-10/1-2010-0025).

Cellulases and pectic enzymes – the armoury of plant pathogen *Colletotrichum coccodes*?

K.E. Hauer(1), R. Willfurth(1), C. Schuhmann(1), W.-D. Lienhart(2), E. Stabentheiner(1), P. Macheroux(2), M. Müller(1)

(1) Institute of Biology, Plant Sciences, University of Graz, Schubertstraße 51, 8010 Graz, Austria
(2) Institute of Biochemistry, Graz University of Technology, Petersgasse 12/2, 8010 Graz, Austria

Cellulases and pectinases are important infection factors that facilitate the process of plant cell wall degradation by some plant-pathogenic fungi, e.g. *Fusarium sp.,* and thus intrusion into the host plant tissue.
C. coccodes causes a potato disease known as black dot and some anthracnose forms at other Solanaceae (e.g. chili and tomatoes). By different means of methods (minimal medium, enzymatic assay and zymogram), it was observed that the phytopathogenic fungus produces extracellular cellulases as well as pectic enzymes [1], [4], [5]. Furthermore, some of the enzymes´ properties (pH optimum, molecular weight, structure) are investigated by protein gel electrophoresis, MALDI (mass spectrometry method) [3], [4] and further assays. Additionally, the distribution of cellulose and pectin within potato tubers are examined by microscopic methods (light microscopy and scanning electron microscopy) [7] and effects of above-mentioned enzymes on potato tissues on cellular level is investigated [2], [3], [6].

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Investigating enzyme activity in some *Pinguicula* species

A. Hamidipour, I. Lichtscheidl-Schultz

Core Facility Cell Imaging and Ultrastructure Research, University of Vienna

The genus *Pinguicula* (butterworts) comprising approximately 100 species is part of Lentibulariaceae family [1]. *Pinguicula* is mostly distributed in the northern hemisphere and in tropical and subtropical America, and is associated with nutrient poor, wet soils. This genus is truely carnivorous in the sense that plants are able to attract, catch, digest and absorb the nutrients from small insects. In *Pinguicula*, insects are caught and digested by two kinds of glandular trichomes, either sessile or stalked, on the upper side of rosette leaves, ''flypaper traps'' [2,4]. It was suggested that the glands have dual function: stalked glands produce mucilage which attracts and catches insects, whereas sessile glands produce digestive enzymes. In addition, movement of leaves was described [3, 4].
Since *Pinguicula* has very big variety in leaf morphology and gland structure, the goal of our work was to revisit leaf movements and glandular functions in different species.
We investigated the movement of leaves in 30 different species. We localized the production of digestive enzymes by break-down of silver gelatin on photographic filmstrips [3], and we tracked the absorption of digested proteins by feeding the leaves with fluorescent proteins [5].

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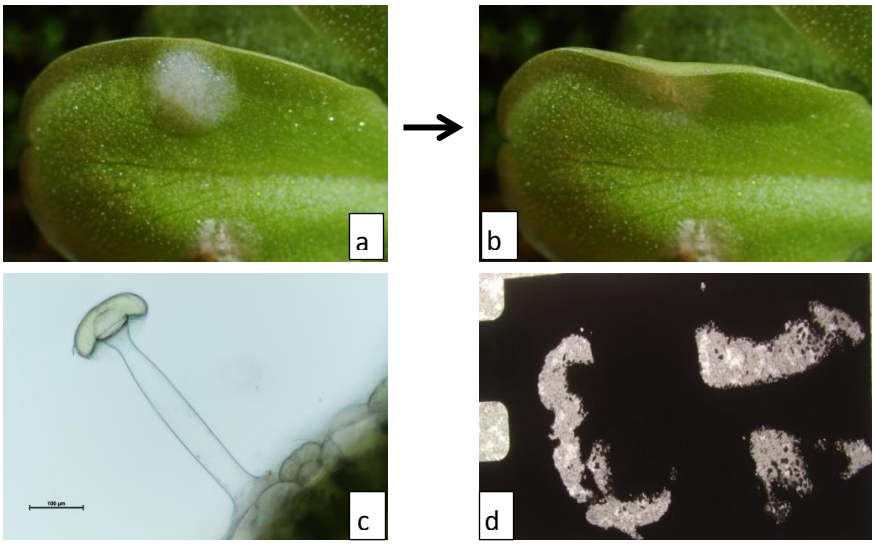

Pinguicula megaspilea: a,b) leaf movement after feeding with BSA, c) sessile and stalked glands, d) enzyme activity detection on photographic film.

Plant pathogen interactions of *Fusarium ssp.* and *Armoracia rusticana* and possibilities for prevention

M. Jančar, M. Müller

Institute of Biology, Plant Sciences, University of Graz, Schubertstraße 51, 8010 Graz, Austria

The incidence and severity of internal discoloration of roots of *Armoracia rusticana* is a severe problem and a serious threat to horseradish production. The internal discoloration of the root begins with dark brown to black discoloration of the vesicular system and gradually spreads to core and cortex areas in the root. Roots with such symptomatic are not acceptable for processing and are discarded. The internal root discoloration is usually followed by root rot. Root discoloration and root rot are mostly caused by *Verticillium, Fusarium, Pseudomonas* and *Erwinia* species [1]. In many cases the internal discoloration and rot on roots of *Armoracia rusticana* is caused by *Verticillium* or *Fusarium* species.
Verticillium species are soil-borne and cause *Verticillium* wilt – a plant disease that affects the vascular bundels of many different hosts. Control of *Verticillium* wilt is difficult, because of the phytopathogens feature to become dormant in the soil for many years in the absence of a suitable pant host [2]. Two common *Verticillium* species causing diseases on *Armoracia rusticana* are *Verticillium dahliae* and *Verticillium longisporum* [1].
Many plants have at least one *Fusarium*-associated disease. Fungi of this species can grow as apparently symptomless endophytes under many conditions. The type of diseases varies intensively in their severity – many of them include root or stem rots, cankers, wilts, fruits or seed rots and leaf diseases [3]. The most common species of *Fusarium* associated with discoloration of horseradish roots are *Fusarium solani* and *Fusarum oxysporum* [1].
Control of this disease has been problematic in many regions. One reason for lack of effective measurements for control of internal discoloration of horseradish roots is an insufficient information about the aetiology of the disease [1]. Before a method for disease prevention or control can be chosen, the pathogen has to be identified, characterized and studied. The pathogen must be recovered from the environment and cultured using different culture media. The identification of the disease-causing pathogen can be approached through different methods regarding their morphological and genetic properties [3].
After the identification of the disease causing phytopathogen an adequate method for disease prevention and control can be selected. Because of the dangerous potential of fungicides for living organisms and the environment, control methods with a biological origin should be taken into account.

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Vegetative and reproductive performance along a climatic gradient does not explain GP in *Ranunculus kuepferi*.

U. Ladinig, J. Wagner

Department of Botany, University of Innsbruck, Sternwartestraße 15, 6020 Innsbruck

Apomictic taxa are mainly polyploid and produce seeds asexually. They often show geographical parthenogenesis (GP) which means that they have latitudinally and elevationally larger distribution ranges than their sexual relatives, and tend to colonize previously glaciated areas more frequently. Various models try to explain this phenomenon, among those polyploidy which might give apomicts a fitness advantage over the diploid parents in stressful habitats. However direct evidence is missing. We therefore pursued an experimental ecological approach by testing the response of the sexual/apomictic model plant *Ranunculus kuepferi* Greut. et Burd. to different climatic conditions. The self-compatible tetraploid apomicts are widespread in the Alps whereas the self-incompatible sexual relatives are restricted to the Western Alps. Along an elevational gradient of 1000 m, experimental plots with apomictic and sexual individuals were established in the subalpine zone, in the lower and higher alpine zone, and in the alpine-nival ecoton in the Austrian Alps. Growth performance and reproductive success were investigated in 3 consecutive years. Additional examinations were carried out in natural sexual and apomictic populations. Growth parameters did not indicate a clear fitness advantage of polyploidy over diploidy in *R. kuepferi*. Diploids tended to develop even more ramets and thus more leaves per individual and showed a higher specific leaf area than tetraploids. Only the rhizome biomass and the number of roots significantly increased with elevation in tetraploids but not in diploids.

Apomictic tetraploids developed slightly more flowers per ramet and about twice as much carpels per flower than sexual diploids. Nevertheless, seed output was higher in diploids than in tetraploids at all sites – mainly due to the high number of malformed ovules.

Overall, for *R. kuepferi*, the study did not confirm the hypothesis that polyploid apomicts show a higher developmental plasticity and therefore cope better with harsh environmental conditions than diploid sexuals. It has to be assessed whether other intrinsic factors such as the capacity for uniparental reproduction can explain the successful spread of the apomicts throughout the Alps.

Ni tolerance and its distinguished amelioration by chelating agents in *B. napus* cultivars

H. Nawaz(1, 2), S. Manhalter(1), A. Ali(3), M.Y. Ashraf(3), I. Lang(1)

(1) Cell Imaging and Ultrastructure Research, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria
(2) Department of Botany, University of Sargodha, Sargodha 40100 Pakistan
(3) Nuclear Institute for Agriculture and Biology, P.O Box 28, Jhang road, Faisalabad, Pakistan

Heavy metal pollution is a problem of ever increasing importance. Among them, Nickel (Ni) has gained considerable attention in recent years, because of its rapidly increasing concentration in soil [1]. Excessive amounts of plant available Ni have negative effects on plant health and growth. This is very well investigated in a wide range of species and crops [2] but there is not as much known about the influence of elevated Ni levels on root anatomy and possible ameliorative effects of chelating agents.
In this study, we utilized light microscopy to observe anatomical changes in canola (*Brassica napus*) roots and investigated the element content by X-ray microanalysis. A Ni tolerant (Con-II) and a Ni sensitive cultivar (Oscar) [3, 4], were selected for this purpose. The plants were treated with 30 ppm $NiSO_4$ right after sowing. Citric acid and EDTA (alone or in combination) were applied two weeks after sprouting, to observe the influence of chelating agents in metal stress amelioration. After 30 days of cultivation, Ni treatment led to increased cortical layers in roots of the tolerant Con-II variety whereas additional EDTA and citric acid treatment reduced the cortex in this cultivar. The sensitive Oscar variety showed no adjustments in the root cortex. According to X-ray microanalyses, Ni ions were more dispersed in the tolerant cultivar. We investigate the hypothesis that a locally enhanced capacity of binding metals to the cell wall allows the plants to tolerate more heavy metals.

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Calcium and iron content in biofilms of active travertine springs in Slovakia

M. Marcinčinová(1), M. Goga(1,2), D.Ručová(1), M. Weidinger(2), M. Bačkor(1)

(1) Department of Botany, Faculty of Science, Pavol Jozef Šafárik University in Košice, Mánesova 23, 040 67 Košice, Slovakia
(2) Core Facility Cell Imaging and Ultrastructure Research, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria

Formation of algal or cyanobacterial biofilm is possible on any moist and illuminated surface, usually in streams or nearby humid environment. In Slovakia, there are multiple travertine springs still active. However, only limited information is available. Three of which (Sivá Brada, Bešeňová and Močiar Stankovany) were selected for this work. This environment is especially suitable for cyanobacteria and diatoms, which form macroscopic biofilm directly in the stream of calcium-rich water. Calcium crystalizes on the surface of the cells or in the biofilm matrix. Common species found at selection sites were cyanobacteria *Phormidium* spp., *Oscillatoria* spp. or diatom *Encynopsis* spp.
Air-dried samples were analyzed by a scanning electron microscope equipped with an EDAX system for performing energy-dispersive X-ray microanalysis (EDX). Following elements were measured: C, O, Al, Si, S, K, Ca and Fe.
There is still not enough data about composition of mineral waters of Slovakia. Our results confirmed high calcium content in biofilms from all three localities. Locality Močiar Stankovany seems to have least amount of minerals overall. Sivá Brada is characteristic of highest calcium content, Bešeňová has less calcium than Močiar Stankovany but also contains highest amount of iron.
The amount of calcium in organisms differs – significant amount can be found in the biofilm matrix or on the cell walls and the mucilage sheath of cyanobacteria, or in the matrix.

Deep supercooling of buds of *Alnus alnobetula*: surface impregnation with lipophilic substances allows innocuous growth of ice masses between leaf primordia

G. Neuner, B. Kreische, D. Kaplenig, K. Monitzer, R. Miller

University of Innsbruck, Department of Botany, Unit Functional Plant Biology, Sternwartestr. 15, 6020 Innsbruck, Austria, Gilbert.neuner@uibk.ac.at

The frost survival mechanism of overwintering vegetative buds of angiosperms is hardly understood. Without experimental evidence it had been suggested to be tolerance of extracellular freezing. The current study assessed the frost survival mechanism of buds of one of the frost hardiest temperate woody angiosperm species, *Alnus alnobetula*, by employment of IDTA (Infrared Differential Thermal Analysis), psychrometry and cryomicroscopy.
In midwinter of 2016/17 the buds achieved a frost resistance of -42,5 °C (LT50). In contrast to earlier suggestions for angiosperms, the buds of *A. alnobetula* could be shown to survive ice free by deep supercooling. An ice barrier prevents penetration of ice from the frozen stem into the bud. No freezing exotherm was detectable when bud primordia were cooled down below the frost killing temperature. Such exotherms would be evoked by intracellular lethal freezing upon breakdown of supercooling. This indicates that the frost killing event in *A. alnobetula* buds is different. It could be excessive freeze dehydration. However, water potentials of in the frozen state excised bud primordia collected during successively lower freezing temperatures did not show any freeze dehydration of primordial cells. Cryomicroscopic investigations revealed a completely new mechanism. Until now, upon freeze dehydration translocated ice masses could be exclusively shown to form in the subtending stem and/or bud scales. Bud primordia of *A. alnobetula* actually get freeze dehydrated, but in contrast to all known locations of formation of translocated ice masses, in this species ice forms inside the bud between the primordial leaves. In supercooling Norway spruce buds contact with surface ice would immediately trigger intracellular freezing of the primordial cells. In buds of *Alnus* species the primordial leaf surfaces are impregnated by a sticky, lipophilic substance which is excreted by glands. They have been analyzed to be triterpenoids and flavonoid aglycones. These substances are suggested to be highly efficient in prevention of extrinsic ice nucleation of the supercooled bud and therefore have antifreeze power.
The buds of *A. alnobetula* show deep supercooling. The primordial cells get freeze dehydrated and water is shifted to ice masses that grow in between of the leaf primordia inside the bud. This is a newly described frost survival mechanism. The antifreeze substances are candidates for application in agriculture where they could be sprayed to plant surfaces to protect from extrinsic ice nucleation and to aid transient supercooling during night frosts.

Phytohormone release by the optionally lichenized alga *Coccomyxa* sp.

G. Pichler(1), W. Stöggl(1), D. Gerna(1), F. Candotto Carniel(2), L. Muggia(2), I. Kranner (1)

(1) Department of Botany, University of Innsbruck, Sternwartestraße 15, A-6020 Innsbruck.
(2) Dipartimento di Scienze della Vita, Università degli Studi di Trieste, Italy.

Evidence is emerging that extracellular phytohormones play important roles in plant-plant and plant-microbe interactions. We aim to resolve open questions regarding the molecular crosstalk between photobionts and mycobionts during lichenization, here defined as the transition from a free-living to a symbiotic stage. We present a new method to detect extracellular phytohormones in exudates of the alga *Coccomyxa* sp. an optionally lichenized photobiont of the lichen *Schizoxylon albescens.* Algae were grown on Teflon filter discs to prevented algal contamination of the solid agar medium underneath. Phytohormones released by algae cultured on 2% agar containing Bold's Basal Medium (BBM) were analysed by liquid chromatography coupled to mass spectrometry (LC-MS/MS). In the freeze-dried agar pellets significant concentrations of salicylic acid and trace amounts of abscisic acid were found, and potential roles of these phytohormones for lichens are discussed. The final goal is to improve our understanding of signalling compounds involved in mycobiont-photobiont interactions.

Acknowledgements. We gratefully acknowledge financial support by the "Office of the Vice Rector for Research" (code 2017/3/BIO-31).

Cold Air Talus Sites in the Eastern Alps

W. Punz

Department of Ecogenomics and Systems Biology (UZA I) University of Vienna 1090 Wien, Althanstraße 14

The term “Cold Air Talus Sites” (“Kaltlöcher”, “Ventarolen” etc.) refers to an aestival efflux of cold air from cavities usually (or possibly even obligatorily) associated with the base of rocky slopes. For centuries this phenomenon has been already known and used, especially in the Alpine regions, to keep milk and other comestibles cool (“Milchhäuser”, “Kühlhäuschen”). An explanation of this effect was given by Balch as early as 1900 who wrote that “*the cold air of winter … permeates the cave, and in course of time freezes up all the water which, in the shape of melting snow or cold winter rain or spring water, finds it way in”* [1]. A recent comment on this topic (together with explanations and examples) is given by Wakonigg [2].
Our comprehensive paper for the Eastern Alps in 2005 listed 43 sites [3]; an update in 2015 provided 83 sites [4], while the most recent publication [5] already reports 107 of such habitats. Itemized by countries there can be found 57 localities in Austria (Carinthia: 17, Lower Austria: 3, Upper Austria: 4, Salzburg: 11, Styria: 10, Tyrol: 8, Vorarlberg: 4), 38 in Italy (Südtirol: 21; Trento: 11; other provinces: 6), 8 in Switzerland, 3 in Germany, 1 in the Czech Republic, and 1 in Slovenia.
An explicit relation between cold air efflux and talus slopes (with a predominant exposition between NW and NE) exists for more than half of all sites. Episodic measurements of air temperature have been conducted on more than one third of all places, while continuous registration of climatic conditions is comparatively rare. No correlation between altitude or latitude and lowest summer temperature could be found. Data on flora and vegetation exist for more than 50% of all habitats: sometimes only a single plant species is mentioned; in contrast, complete phytosociological tables are given for other sites. Zoological informations have been collected from approximately one tenth of all localities documenting the role of cold air taluses as hot spots for endemites and Red List species, especially for sites at low altititude; in some cases, local glacial relic populations could be found. A noteworthy high percentage (more than a third of all sites) of human utilisation (both historical and recent, including touristic purposes) has been documented.
Cold air vents of the talus slopes may warm under global climate change conditions. Documentation of these sites may contribute to the indication of such changes (see e.g. [6]) and might help to take measures to preserve these ecological interesting and valuable habitats.

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Can sulfur fertilization improve the defense reaction of the Styrian oil pumpkin after *Didymella bryoniae* treatment?

K. Reibenschuh, E. Stabentheiner, M. Müller

Institute of Biology, Plant Sciences, University of Graz, Schubertstraße 51, 8010 Graz, Austria

The Styrian pumpkin seed oil, also known as “black gold”, not only convinces with its taste, but also finds application in medicine [1]. This oil is traditionally extracted from the seeds of the Styrian oil pumpkin, which explains why the Styrian oil pumpkin (*Cucurbita pepo* L. *styriaca*) is one of the most important crops in Styria. Unfortunately, the number of pathogens that infest the Styrian oil pumpkin has risen dramatically in recent decades [2]. One of these pathogens is the fungus *Didymella bryoniae*, which was first identified in Styria in 2004, and can be found in every pumpkin patch [3]. This necrotic fungus causes diseases like the “gummy stem blight”, leaf drought or fruit rot, which is also known as “black rot”, and leads, under favorable conditions, to enormous crop failures [4]. One way to reduce such huge crop failure is to strengthen the plant through special fertilization, because it is a well-known fact that sulfur intake has a positive effect on the natural pathogen defense of plants [5]. The aim of this master thesis was to show that sulfur fertilization strengthens the plant´s immune system and at the same time improves the plant´s defense against pathogens. Results of these experiments confirmed that plants which were treated with sulfur displayed rapid development, as well as large dark green leaves and attenuated disease symptoms. Furthermore, stress indicators such as chlorophyll fluorescence, photosynthesis rate and the chlorophyll content of leaves were determined and measured several times during the course of the experiment. Despite *Didymella bryoniae* infection, sulfur-treated plants indicated positive effects concerning the measured stress parameters. Moreover, biochemical analyzes, such as the determination of pigments and antioxidants like glutathione and α-tocopherol, were executed. At this point, positive effects on sulfur- fertilized and *Didymella bryoniae* infected plants were observed.

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Distinguishing the mechanistic contributions to NPQ in *Chlamydomonas reinhardtii*

T. Roach, C.S. Na, I. Kranner

Department of Botany, University of Innsbruck, Austria

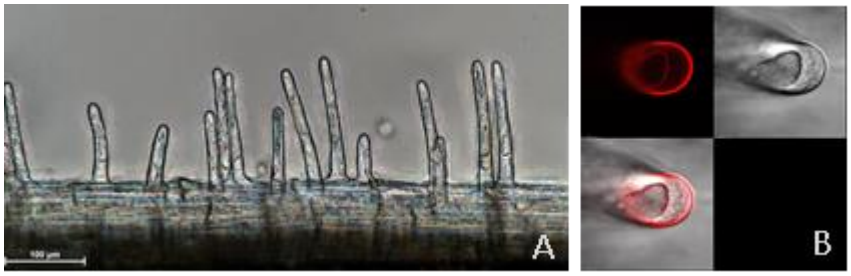
Photosynthetic organisms have to tolerate rapid changes in light intensity, which is facilitated by non-photochemical quenching (NPQ) and involves modification of energy transfer from light-harvesting complexes (LHC) to the photosystem reaction centres. Mechanisms of NPQ include dissipating excess light energy to heat (qE) and the reversible attachment of LHCs to photosystems (state transitions / qT), which are considered separate NPQ mechanisms. The contribution of the xanthophyll cycle to qE in *Chlamydomonas reinhardtii* is less than in other alga species and plants, whereas the LHCSR3 protein has a clear role in qE and photoprotection. Here, it is shown in the *npq4* mutant, deficient in LHCSR3, that energy coupling from the LHC to the photosystems (PSII and PSI) during qT is also disrupted. Stt7-mediated phosphorylation is required for qT, but no major differences in LHC phosphorylation levels or LHC compositions were found in *npq4* compared to wild-type cells. Further inclusion in the study of *stt7*, that is absent in Stt7 kinase, showed that LHCSR3 is involved in the rapid qT transitions (<2 min), whereas Stt7-mediated kinase activity corresponds with the slower qT transitions (up to 10 min). It is concluded that NPQ in *C. reinhardtii* has a much greater mechanistic overlap than previously recognised.

Heavy metal uptake via endocytosis during vesicle turnover in growing root hairs of *Triticum aestivum*

V. Romagna, I. Lichtscheidl

Cell Imaging and Ultrastructure Research, Faculty of Life Sciences, The University of Vienna, Austria

Root hairs are single cells with great importance in nutrient uptake, and they function in anchoring plants in the soil [1]. Zinc in the soil is needed for the metabolism of plants, but in high concentrations it becomes toxic: it causes reduction in growth, performance and yield and can lead to the death of the plant [2]. Root hairs grow by tip growth, involving vesicle trafficking, cell secretion and cell wall modifications [3, 4]. During exocytosis of pectins some vesicles become integrated into the plasma membrane, whereas others follow the "kiss-and-run" model and return to the cytoplasm [5]. During recycling of such vesicles, plants might be able to take up substances like heavy metals from the surrounding media into the vesicle, which transport their content back into the cell. Furthermore, root hair morphology can be affected by different factors including heavy metal impact, cells show thickened cell wall and curving or branching pattern [6].
We chose *Triticum aestivum* for our investigations as it represents an economically important plant with linkage to the food chain. Seedlings are cultivated in a ½ MS solution with different zinc concentrations. Root hair elongation and branching, as well as the dynamic properties of vesicles during cell wall deposition are investigated by live cell imaging. Fluorescent dyes NewPort Green (zinc visualization) and FM4-64 (plasma membrane staining) are used for localizing zinc and endocytotic vesicles (wide field and confocal fluorescence microscopy); to test for colocalization of zinc in vesicles both dyes get applied simultaneously. Scanning Electron Microscopy and Electron Dispersive X-ray Spectroscopy-analyses are used to determine zinc accumulation in cell wall at the growing tip of root hairs.



A: developing root hairs in *Triticum aestivum*;

B: staining of plasma membrane with FM4-64 by confocal scanning fluorescence microscopy

The diameter of the root hairs is ca. 15 µm.

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Does usnic acid influence ploidy levels in mosses?

D. Ručová(1), M. Goga(1,2), V. Kolarčik(1), M. Sabovljević(3), M. Bačkor(1), I. Lang(2)

(1) Department of Botany, Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafárik University, Mánesova 23, 041 67, Košice, Slovakia
(2) Core Facility Cell Imaging and Ultrastructure Research, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria
(3) Institute of Botany and Botanical Garden, Faculty of Biology, University of Belgrade, Takovska 43, 11000 Belgrade, Serbia

Bryophytes represent the earliest green plants to inhabit terrestrial environment. Adaptation to various habitats during phylogeny make bryophytes ideal organisms to explore the role and biological significance of endopolyploidy. Endopolyploidy is defined as the existence of various ploidy levels in the same individual create by endoreduplication and appears when DNA replication is not followed by mitosis. In general, nuclei with one chromosome set (1C) are monoploid, with two (2C) are diploid and those with three or more chromosome sets are defined as polyploid [1]. The substrate of lichens and bryophytes is quite similar resulting in a competition for space, light and nutrients [2]. Lichens contain various secondary metabolites, and most of them occur exclusively in these symbiotic organisms. They may allelopathically interact with neighboring bryophytes, vascular plants, fungi as well as other lichens. In the present study, we observed level of ploidy in mosses influenced by usnic acid as secondary metabolite. Mosses *Physcomitrella patens* and *Pohlia drummondii* were cultivated for 5 weeks under laboratory conditions and treated with usnic acid at three selected doses (control, 0.01mg/disk, 0.1mg/disk). They were analyzed for the level of endopolyploidy using flow cytometry. Application of usnic acid changed the nuclei proportion and significantly enhanced the endoreduplication index in both tested mosses. In moss *Physcomitrella patens* when mixture contained usnic acid, we observed presence of 2C, strongly evidenced 4C nuclei and 8C nuclei were also noticed. This is reflected into increasing endoreduplication index. Application of usnic acid led to increase of 2C nuclei proportion and significant increase endoreduplication index in *Pohlia drummondii.* Both moss species can be considered as endopolyploid, where the level of ploidy is influenced by secondary metabolites of lichens.

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Impact of drought stress on the cell wall design of larch trees

N. Sasani(1), B. Prats-Mateu(1), L. Paques(2), O. Brendel(3), S. Rosner(4), N. Gierlinger(1)

(1) Institute for Biophysics, BOKU-University of Natural Resources and Life Sciences, Vienna, Austria.
(2) INRA-Unité AGPF, Orlean, France
(3) Université de Lorraine, AgroParisTech, Inra, Silva, Nancy, France
(4) Institute for Botany, BOKU-University of Natural Resources and Life Sciences, Vienna, Austria.

Wood formation is known to be highly influenced by the environment and thus, climate change will have an impact on tree growth. As drought is becoming more and more a limiting factor for tree growth a better understanding of the tree response is needed. In this study two to four year old trees of three different larch species (European-, Japanese- and hybrid larch) were grown under two water regimes: a control group with normal irrigation (26.35% soil volumes' humidity) and a "stressed" group with less amount of water (10% soil volumes' humidity). So far, most of the studies on the impact of drought have addressed variation in tree ring width, wood anatomy and/or hydraulic parameters, but only a few reports have dealt with the impact of drought stress on changed distribution of wood polymers. Therefore we apply for the first time the Raman imaging approach to reveal the position resolved molecular composition of the cell wall on trees grown under artificial drought stress. Microsections were cut from the three species, grown under normal and stressed conditions, and light microscopic stitching images from the whole stem were acquired. By this first step changes in tree growth of the last ring as well as compression wood areas were identified, before selecting appropriate regions for Raman imaging. The preliminary analysis showed smaller tree rings and thus less growth in the drought stressed trees, especially for European and Japanese larch. The Raman analysis will reveal if the slowed down growth also affects the chemical composition of the cell wall.

This work is supported by the START Project [Y-728-B16] from the Austrian Science Fund and by the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement n° 284181 ("Trees4Future").

Yeast in bioremediation – experiments with *A. arenosa* on zinc contaminated soils

S.-S. Sidra, M. Weidinger, I. Lang, I. Lichtscheidl

Core Facility Cell Imaging and Ultrastructure Research, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria

Pollution by heavy metals has negative effects on humans, animals, plants and microbial-communities. High doses of heavy metals in soil can reduce its fertility and hence pose a crucial risk to the cultivation of crops. So-called heavy metal plants can prevail under these toxic conditions; they therefore play an important role in bio-remediation of contaminated soils and the re-establishment of balanced heavy-metal levels that are suitable for non-heavy metal plants and non-resistant microorganisms. However, not only the plants but also the soil-microbial organisms should be regarded as an opportunity for new ways of bioremediation.
In this thesis, *Arabidopsis arenosa,* a heavy-metal plant with a pronounced tolerance to zinc, and a wild-type yeast strain, detected on an *A. arenosa* cultivation, were coupled in various toxicity experiments to determine mutual effects on heavy-metal tolerance.
The experiments show that the wild-type yeast can sustain high levels of zinc (up to 4 mM). Elemental analysis shows a possible accumulation of zinc in yeast cells, which would further point to temporary removal of zinc from the soil. Yeast therefore could be used as a tool to decrease zinc levels in contaminated soils which would empower plants to germinate and grow in. A combined approach of using heavy-metal plants and soil-microorganisms can broaden our view on how to tackle pollution by heavy metals in the environment.

Acknowledgement:
The strain of wild-type yeast was characterized by Katarzyna Turnau.
We thank our gardeners, Thomas Joch and Andreas Schröfl, for their assistance in plant cultivation.

Testing viability of plant cells: can loss of turgor potential signalize cell death

M. Stegner, G. Neuner

University of Innsbruck, Department of Botany, Unit Functional Plant Biology, Sternwartestr. 15, 6020 Innsbruck, Austria, matthias.stegner@uibk.ac.at

Assessment of viability of plants cells can be a big challenge as in certain plant tissues the available methods are either elaborate, yield only ambiguous results or even fail. In an intact plant cell the total water potential (Ψt) is composed of the osmotic potential, the cell wall pressure (turgor) and the matric potential. Upon damage to the cell membranes the turgor pressure collapses. Hence, we hypothesized that by determination of the turgor pressure of cells, cellular damage should possibly be unambiguously be detectable.
Before, during and after exposure to simulated night frosts with different severity, we tried to assess critically low temperature thresholds for frost damage by psychrometric measurement of the water potential of *Viola sp.* leaves.
The turgor pressure of intact *Viola sp.* leaves was determined to be 0,28 ± 0,04 MPa. Frost exposure to -3 °C, -4 °C and -5 °C did not lead to a significant water potential change. In contrast, after exposure to -6 °C and -12 °C, due to loss of turgor, a significant decrease of water potential was recorded. This decrease was similar to the measured turgor, i.e. 0,30 ± 0,06 (-6 °C) and 0,31 ± 0,05 (-12 °C) MPa.
Additionally, ice nucleation in the leaves could be detected with the psychrometric method at -5,6 ± 0,3 °C as water potential of ice is lower than the water potential of liquid water at the same temperature.
A LT_{50}-value calculated based upon changes in water potential with decreasing night frost temperature was -5,3 ± 1,2 °C. A classic viability test using chlorophyll fluorescence yielded a LT_{50}-value of -5,1 ± 0,3 °C. This good agreement underlines the suitability of water potential measurements for determining viability of plant tissues.
Especially for plant tissues that are highly challenging with respect to viability assessment, such as stem tissues, e.g. xylem parenchyma, or seeds, the psychrometric method could be a valuable tool. What is more, due to the immediate response (breakdown of turgor) no latent period for testing is required.

This research was funded by the Austrian Science Fund (FWF-project 30139-B32).

Trichomes on flowers of *Lavandula angustifolia*

G. Strauß(1), G. Raspotnig(2), E. Stabentheiner(1)

(1) *Institute of Biology, Plant Sciences, University of Graz, Schubertstraße 51, 8010 Graz, Austria*
(2) Institute of Biology, Zoology, University of Graz, Universitätsplatz 2, 8010 Graz

Members of the Lamiaceae are characterized by the occurrence of various types of non-glandular and glandular trichomes on leaves, stems and flowers. These glandular trichomes are most significant for fragrance, cosmetic and food industry. Whereas trichomes on leaves and calyx are intensively investigated, trichomes on petals have often been neglected in these studies.
Trichomes on flowers (calyx and petals) of lavender (*Lavandula angustifolia* Mill.) were in the focus of this study. Occurrence, distribution and types of trichomes were investigated using light and scanning electron microscopy. Histochemical tests (sudan black, fluorol yellow, NADI, natural products reagent, vanillin-hydrochloric acid) identified the main products of the glandular trichomes (lipids, terpenes, flavonoids, polyphenolics) and GC-MS-analyses of hexane extracts (leaves, petals) gave first information on mono- and sesquiterpenes produced by these trichomes [1, 2].
The peltate trichome type observed on the outer side of the calyx and the petals was characterized by a basal cell, a short unicellular stalk and a head of four or eight secretory cells with a large subcuticular space. Terpenes and lipids were detected in the subcuticular space, and flavonoids and polyphenolics in the secretory cells. Three types of capitate trichomes could be identified on the flower structures with positive reactions for lipids, terpenes, flavonoids and polyphenolics. A long stalked trichome with prominent warty knobs, a distinct neck cell and a one-celled spherical head producing sticky exudates could be exclusively observed on the inner surface of the petals. The pattern of mono- and sesquiterpenes differed between leaves and petals and also revealed day-time dependent differences.

The study highlighted the trichomes on the flowers of lavender and provides a basis for further studies.

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Sensitivity loss of *Venturia inaequalis* against the plant protection product Delan WG and its effect on the germination behaviour of the conidia

L.M. Strobl, M. Müller, A.C. Waibel, S. Monschein

Institute of Biology, Plant Sciences, University of Graz, Schubertstraße 51, 8010 Graz, Austria

Apple scab caused by *Venturia inaequalis* is the most important disease of apple in Austria. This phytopathogenic fungus has a high risk to form resistance to fungicides [1]. This study investigates whether there is a loss of sensitivity of *V. inaequalis* to the often used protective contact fungicide Delan WG, with the active substance dithianon in the Styrian orchards. This fungicide acts non-specifically in several places in the metabolism in the fungal cell, which should actually result in a low risk of resistance. [2]

For this purpose, young scab-infected apple leaves were collected from two extensive, one biological and six integrated managed locations in Styria in June 2017. One of the two extensive locations was located outside the Styrian apple-growing region in Western Styria and has never been treated with fungicides or other pesticides. It is used as a reference to the remaining locations, which were located in the Styrian apple growing area.

Conidial suspensions were prepared to examine the germination behaviour of the conidia at different fungicide concentrations. Therefore the germination rates of the conidia were determined and the efficiencies of the fungicide concentrations were calculated [3]. The germination behaviour of the conidia within, between and compared to the untreated reference location were investigated.

At all locations, the recommended application concentration of Delan WG reached an efficiency of almost 100 %. Efficiencies of 97 % to 100 % were still achieved at 1:10 dilutions. The lowest dilution (1:1000) showed different results, ranging from efficiencies of 20 % to over 90 %.

With these *in vitro* experiments it can be stated that there is no loss of effect of Delan WG over *V. inaequalis*. However, the different results from the dilutions of the fungicide may already indicate a slow loss of sensitivity of the fungus at some orchards. To confirm this, further investigations are needed. The sensitivity of *V. inaequalis* to Delan WG should be monitored over the next few years to find out if there is any change in sensitivity. Furthermore, investigations on different strains within an orchard would be conceivable.

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Following mulberry (*Morus sp.*) footprints: Geographic distribution and diversity of leaf morphology of heritage trees in Slovenia

P. Sušnik(1), M. Felicijan(1), A. Paušič(1), J. Rabensteiner(2), A. Urbanek Kranjc(1)

(1) Faculty of Agriculture and Life Sciences, University of Maribor, Slovenia
(2) Institute of Plant Sciences, University of Graz, Holteigasse 6, A-8010 Graz, Austria

White mulberry (*Morus alba* L., Moraceae) was brought to Europe from Southeastern Asia in 12th century since the leaves were used as the silkworm's feed in order to establish a functioning European sericulture. It was introduced to Slovenia into the Goriška region during the 16th century, where silk was produced for the Venetian market [1]. From there, the cultivation of mulberries spread towards other regions. Sericulture had reached its peak in the mid-18th century, however since then it was in steady decline because of problems with late-frost die-backs and bacterial diseases of silkworms and trees. Until the 1960s the silk-producing industry lost its importance in Europe caused by the predomination of Asian silk production. Over the centuries the trees became a characteristic element of the cultural landscape within the silk-producing regions and represent a still living cultural and natural heritage of sericulture. Their distribution records are incomplete and in general poorly documented [2,3,4]. The aim of the presented research was to collect data regarding geographical locations of historical mulberries and to define their morphological variability. We recorded exact GPS locations of more than 600 mulberry trees and collected leaf samples during field excursions to different regions of Slovenia during the years 2015-17. The highest density of trees was recorded in Coastal-Karst and Goriška region, followed by Pomurje and Southeast Slovenia. Wide variation in leaf morphology was observed within the species. Eight leaf morphometrical parameters were measured (leaf area, peduncle length, leaf length, leaf width, left and right leaf width, length of left and right basal vein). The shape and size of the leaf may vary according to the age of the plant, position within the canopy, pruning management, the period of growth and multiple environmental factors [5]. Plants react to water stress by reducing their leaf area [6] and individuals from regions with significantly lower rainfall would thus be expected to have smaller leaves. The principal component analysis (PCA) allowed us to establish the most suitable differentiating traits, to closer define morphotypes, and to present correlations between measured parameters with respects to pruning management and geographical distribution. The genotypes included in these morphometric analyses are part of a broader ongoing genetic analysis, which will provide additional insight into the variability of the oldest *Morus alba* genotypes in Slovenia.

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Microscopic-analytical characterization of different sweet potato varieties (*Ipomoea batatas*)

L. Teuschler, M. Müller

Institute of Biology, Plant Sciences, University of Graz, Schubertstraße 51, 8010 Graz, Austria

Ipomoea batatas, commonly called sweet potato, belongs to the Convolvulaceae family (morning glory). Sweet potato is ranked the most important food crop after rice, wheat, potato, maize, and cassava [1]. Originally, from Mexico, it has more or less spread throughout the world and is now grown in more than 100 countries around the world [2].
In this study, differences in the morphology of different sweet potato varieties *(Ipomoea batatas* L.) were examined by means of the microscope. In addition, variations in the chemical composition, especially soluble sugars and vitamin C, of four selected sweet potato varieties after three different preparation methods (raw, steamed, and baked) were investigated by HPLC-analyses.
The characteristic features of the groups orange, Japanese, purple and white regarding peel and parenchyma color, colorant distribution, size and shape of the starch granules and occurrences of latex vessels and calcium oxalate crystals were determined. In brightly exposed tubers, chloroplasts were detected. The sprouting of a halved sweet potato was documented over a period of about 45 days.
Both the total sugar content and the ratio of the individual soluble sugars glucose, fructose, sucrose and maltose were determined after the three preparation methods by means of HPLC. Likewise, the ascorbic acid content was analyzed. In addition, the water content and dry matter content of the raw tubers were calculated.
In conclusion, this work suggests that both the method of preparation and the basic genetics have a significant impact on the sugar profile and sweet taste on the one hand and on the vitamin C content of processed sweet potatoes on the other hand.

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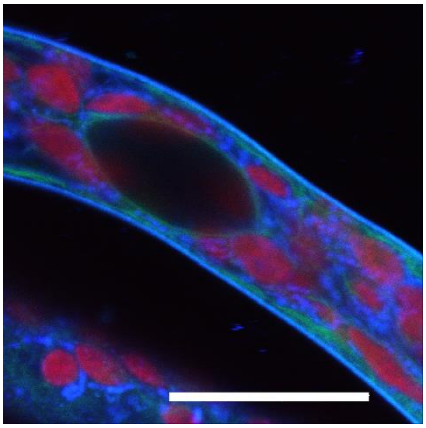
The organization of some subcellular structures in the model moss *Physcomitrella patens*

K. Thiering, I. Lang

CIUS, Core Facility Cell Imaging and Ultrastructure Research Althanstraße 14 (UZA I), 1090 Wien, katrin.thiering@gmx.at

We investigated different subcellular structures in the tissues of three *Physcomitrella patens* GFP-lines. The ER me-GFP line shows the endoplasmatic reticulum (ER) marked with GFP, the Life-Act me-GFP line has labelled actin microfilaments and the Tub me-GFP line labelled tubulin.
Confocal microscopy was used to detect the respective subcellular structures in gametophore and protonema cells. Specific dyes were used to confirm these structures in wild type tissue. The membrane specific styryl dye FM 1-43 showed the same structures as the ER me-GFP line, but was additionally found in vesicles and the plasma membrane. Plasmolysis was used to investigate the effect of osmotic stress on the plant cells and to detect possible changes on the subcellular structures. After plasmolysis, the Hechtian strands [1] could be seen in the ER-GFP mutant and the sheets in the ER decreased.
In ongoing analyses, we compare the cell lines with two other moss species, *Mielichhoferia elongata* (Bryales) and *Pohlia drummondii* (Bryales), as well as seed plants like *Arabidopsis thaliana*.



Confocal image of protonema cell. ER-GFP (green), membranes (blue, FM 1-43), chloroplasts (red). Scale bar: 20 µm.

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We thank Dr. Magdalena Bezanilla for providing the cell lines.

Uncovering the role of the single berberine bridge enzyme homolog of *Physcomitrella patens*

M. Toplak(1), G. Wiedemann(2), J. Ulićević(1), B. Daniel(1),1 S.N.W. Hoernstein(2), J. Kothe(2), J. Niederhauser(1), R. Reski(2), (3), A. Winkler(1), P. Macheroux(1)

(1) *Institute of Biochemistry, Graz University of Technology, Austria*
(2) *Plant Biotechnology, Faculty of Biology, University of Freiburg, Germany*
(3) *BIOSS Centre for Biological Signalling Studies, University of Freiburg, Germany*

The berberine bridge enzyme- (BBE-) like protein family is a large enzyme family found in bacteria, fungi and plants. It is named after its best characterized member, the berberine bridge enzyme from *Eschscholzia califonica* (*Ec*BBE), which is a key enzyme of the benzylisoquinoline biosynthesis, where it catalyses the important cyclization reaction of (*S*)-reticuline to (*S*)-scoulerine. Interestingly, even very basal plants that do not have any biosynthetic pathways for alkaloids, encode BBE-like genes, which raises the question of the primordial role of BBE-like proteins in plants.
With the goal of getting a better understanding of the evolution and development of the whole family of BBE-like proteins, we initiated the structural and biochemical characterization of the BBE-like enzyme from *Physcomitrella patens* (*Pp*BBE), which is the most basal plant possessing a *BBE-like* gene. We found that the enzyme exhibits very similar structural and spectral features as previously shown for other BBE-like proteins, whereas its catalytic role was found to be rather different. Using screening assays we could show that *Pp*BBE acts on the disaccharide cellobiose, which gets oxidized at the anomeric carbon to yield the corresponding lactone as product. With the help of the a structure guided mutagenesis program we were in the following able to reveal the role of various active site residues in catalysis, which further enabled us to postulate a reaction mechanism.
Our *in vitro* findings are further supported by the *in vivo* results obtained from the characterization of a *Pp*BBE knock-out strain. It was found that the enzyme is highly expressed in the chloronema phase, an early stage of the plant's life cycle, where carbohydrate metabolism is strongly upregulated and that the protein is secreted to the extracellular space. Hence, it is possible that *Pp*BBE is involved in the later steps of cellulose degradation, thereby allowing the moss to most efficiently use cellulose for energy production.

Morphometric analyses of phytoliths in different mulberry genotypes

T. Ugulin(1), A. Urbanek Krajnc(2), G. Zellnig(1), M. Müller(1)

(1) Institute of Biology, Plant Sciences, University of Graz, Schubertstraße 51, 8010 Graz, Austria
(2) Faculty of Agriculture and Life Sciences, University of Maribor, Pivola 10, 2311 Hoče, Slovenia.

Many plant groups are known to deposit opal silica (SiO_2) within and between cells and tissues in solid form creating amorphous structures commonly known as phytoliths or silica bodies. They are essential for growth, mechanical strength rigidity, predator and fungal defense, stiffness and cooling. Plants attacked by herbivores tend to accumulate more silica in their leaves than non-attacked plants, and higher level of silicification is associated with lower herbivory [1]. Phytoliths are of vital importance for the fields of taxonomy, paleoecology, and archeobotany [2], They have a defined anatomical shape and allow distinction among taxonomical groups. Furthermore, they are highly durable under a wide range of depositional conditions. Silica nanoparticles have proven to be important for several biotechnological and biomedical applications such as biosensor design, drug delivery, cell labelling etc. [3]. In last decade, mulberries became model organisms with respect to phytolith formation in cell walls [4,5]. Silica opals in mulberry leaves offer complementary features as they can be used as byproducts in sericulture. The question arises whether morphometric variables associated with size are sufficiently reliable for taxonomical distinction among mulberry taxa, and whether the size increases with ploidy level.
In the present research, phytolith number, size (area, perimeter) and shape (convexity and roundness) among 19 mulberry taxa were analyzed with the respect to idioblast's size. Microscopical investigations were performed on Olympus microscope (Provis AX 70) equipped with a 100 W mercury arc lamp under UV fluorescence by applying semi-automatic image analysis.
The results showed quantitative and qualitative differences amongst different taxa, which depended on their ploidy level and were significantly correlated with idioblasts' size. The largest phytolith area was determined in leaves of *Morus nigra*, a polyploid species with 2n = 22x = 308. Significant differences were also obtained between closely related taxa. We used this preliminary study to explore, which attributes can be used as reliable in taxa evaluation. As the results of this preliminary study showed significant differences, the presented morphometrical traits will be used in further evaluations of the old mulberry varieties, which can be found in the former silk-producing regions.

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Winter snow cover is essential for flower bud survival in high mountain plants

U. Ladinig (1), K. Gruber(1), O. Buchner(2), G. Neuner(1), J. Wagner(1)

(1) Department of Botany, University of Innsbruck, Sternwartestraße 15, 6020 Innsbruck
(2) University of Salzburg, Hellbrunnerstraße 34, Department of Biosciences, 5020 Salzburg

The majority of perennial high mountain plants initiates flower buds in the year before anthesis which, depending on the species, pass winter in different developmental states from early primordial to highly differentiated. High mountain plants usually pass winter below the snow at temperatures between 0 and -5 °C. Growing on rocks and windblown ridges, however, they experience free air temperatures which can reach -25 to -30 °C. We know from numerous studies that vegetative organs are usually sufficiently hardy to survive such temperatures, but almost nothing is known about the winter frost tolerance of reproductive structures. In the present study, we investigated winter frost tolerance of dormant flower buds in three common plant species in the European Alps with different site preferences: the cushion plants *Saxifraga bryoides* L. (subnival-nival) and *Saxifraga moschata* Wulfen (alpine-nival) growing on scree and solid rock with partly little snow cover in winter, and the nival species *Ranunculus glacialis* L. preferring snow-rich sites. Plant individuals were collected at the natural sites at the end of the growing season, potted in alpine soil and kept at a subalpine location until the start of the experiments in January. Potted plants were exposed to short-term (ST, one night) and long-term (LT, one week) frost between -10 and -30 °C in temperature-controlled freezers. Reproductive buds were isolated and examined for frost damage using vital staining. In addition, ice nucleation temperatures in the short-stem shoots of the cushion plants were recorded by IDTA (Infrared Differential Thermal Analysis). A part of the individuals were returned to the subalpine site and, at the time of anthesis, flowering frequency was determined.
The IDTA-analysis showed that short-stem shoots of both saxifrages did not supercool (cooling rate 7 $K.h^{-1}$): all shoots were frozen at -5 °C in *S. bryoides*, and at -7 °C in *S. moschata*. Flower bud vitality decreased significantly with temperature and exposure time in both species. Already -10 °C LT-frost caused initial frost damage in floral apices. Below -20 °C the proportion of vital apices was about 30% (ST) and 3% (LT) in *S. bryoides*. The losses were less pronounced in *S. moschata*: at -25 °C on average still 70% (ST) and 50% (LT) of floral apices appeared vital. Flowering frequency essentially reflected the results from the vitality test in so far as already moderate frost substantially decreased the percentage of flowering short-stem shoots. However, due to a high variability among individuals, the differences between the temperature levels were not significant. In *R. glacialis*, vegetative and reproductive buds overwinter below ground. Temperatures down to -20 °C did not cause notable damage during ST-frost but significantly reduced regrowth after LT-frost. ST- and LT-treatment at -30 °C killed the plants completely.
We could clearly show that flower buds of high mountain plants are susceptible to frost in winter. It is to be expected that without sufficient snow cover a part of the flower buds do not survive winter which reduces flower abundance and thus the reproductive output in summer. This problem gains particular relevance in the context of winter periods with low precipitation and winter warming events leading to the melting of the protective blanket of snow.

Reduced sensitivity of *Venturia inaequalis* against a multi-site fungicide tested in different apple orchards in Styria, Austria

A. Waibel, L. Strobl, S. Monschein, M. Müller

Institute of Biology, Plant Sciences, University of Graz, Schubertstraße 51, 8010 Graz, Austria

The pathogen of apple scab, *Venturia inaequalis*, is combated through applications of different fungicides including Merpan® 80 WDG (ADAMA Germany GmbH). A loss of sensitivity of the pathogen regarding this fungicide was observed and reported by Styrian farmers. The active component of this preventative multi-site fungicide is the phthalimide Captan, which interacts with thiol-groups [1]. Another paper mentions signs of decreased sensitivity of *V. inaequalis* isolates against other multi-site fungicides [2]. The aim of this study was to examine the current sensitivity status of *V. inaequalis* against Merpan® 80 WDG in Styria, Austria.

The germination ability of conidia from nine locations across Styria was analysed. Three locations are biologically grown orchards whereas one is not exposed to any chemical treatment and therefore is used as reference. The remaining six locations run conventionally where Merpan® 80 WDG is applied to the apple trees. All locations except the isolated reference location are situated in the south-eastern pomiculture area of Styria.

In the experiments, spore suspensions of each sample were produced and treated with the recommended fungicide application concentration (875 ppm Captan) as well as a tenth, a hundredth and a thousandth thereof. After incubation in darkness in multiwell plates for 24 hours at 20 °C, the germination status of at least 200 spores per sample and concentration was visually determined [3]. The effectiveness of the fungicide concentrations in each location was calculated referring to the results of untreated controls [4]. All locations were compared amongst each other and in particular with the reference location in the final analysis.

Results indicate that the recommended application concentration of Merpan® 80 WDG still is highly effective in all locations. Similarly, conidia from the reference location nearly did not germinate at all even in a thousandth of the recommended fungicide concentration. Anyway, this fungicide level generated extremely variable results amongst the other locations. This leads to the assumption that the sensitivity of *V. inaequalis* against Captan slowly but successively decreases in the fungal populations of divers Styrian pomiculture areas.

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Unravelling hierarchical microstructure and chemical changes of walnut shells

N. Xiao, P. Bock, N. Gierlinger

Institute of Biophysics, University of Natural Resources and Life Sciences, Vienna, Austria

In a common sense a nut is a fruit composed of a hard shell and a seed. The nutshells give protection to the seeds with the embryo, which are crucial for the next tree population. A detailed understanding of the microstructure and the chemical changes is the basis for identifying the structural features that are most important for answering the question on how nutshell design is optimized to bring up that fascinating life protecting materials.
In this study extensive characterization of the walnut shell was carried out by applying Fourier transform infrared (FT-IR) microscopy and Raman microscopy. Revealing intrinsic morphological principles helped to highlight the exact dependencies between different tissues, composed of different sclerotic cells, arranged in a specified manner. The identification of chemical compounds within different tissues and cells was performed by using different multivariate data analysis methods, such as cluster analyses and principal component analysis (PCA). With the help of cluster analysis, similar spectra from the FT-IR were grouped to clusters, which differentiated functional groups and the corresponding chemical composition. Additionally unmixing multivariate data analysis approaches, such as non-negative matrix factorization (NMF) and vertex component analysis (VCA) have been applied to find the most significant chemical components and their distribution throughout the measured area. Our results clearly indicate the potential of Raman and infrared micro spectroscopy to gain new insights into chemical changes of the nutshell microstructure of different development stages of walnut. In the long term we aim at revealing important structure-function relationships of the nutshell design during development to result in possible applications in biomimetic research.

“This project has received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation programme (grant agreement No [681885])”.

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